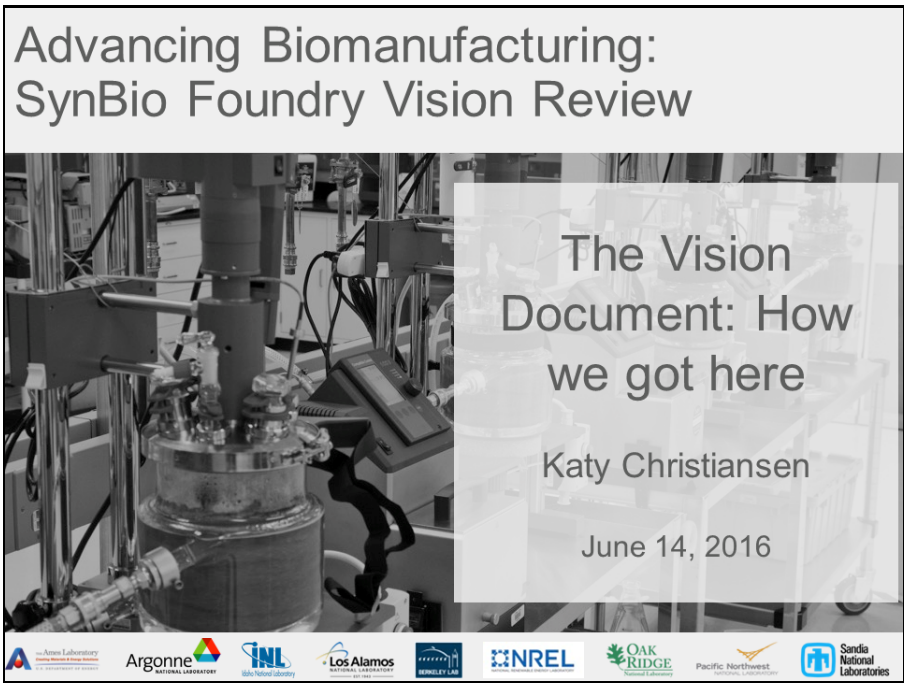
Agile BioFoundry Vision Workshop

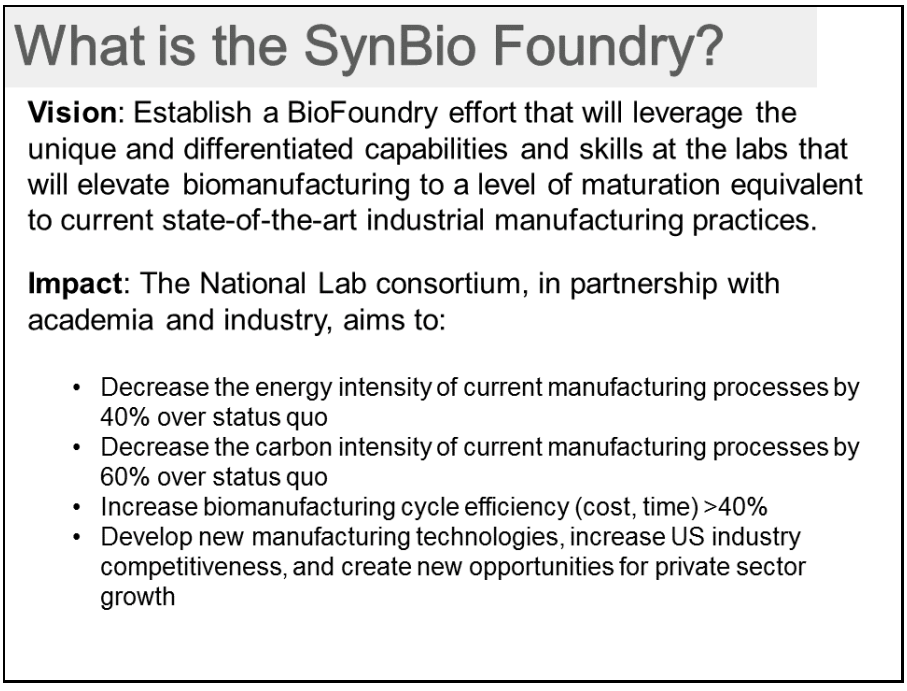
June 14, 2016

ROSTER

LyinDSC	THINKER	drldhsealll@gmail.com
ShawnJones	THINKER	sjones@whitedoglabs.com
Amit-Solvay	THINKER	sehgal.email@yahoo.com
Phil Laible	THINKER	laible@anl.gov
leslie.ovard	THINKER	leslie.ovard@inl.gov
b123	THINKER	brendan.deveney@hq.doe.gov
Lori	THINKER	lgiver@calysta.com
John Cumbers	THINKER	john.cumbers@synbiobeta.com
Babs Marrone	THINKER	blm@lanl.gov
Andrew	THINKER	aconley@lygos.com
Taraka Dale	THINKER	tdale@lanl.gvo
HLiao	THINKER	hans_liao@cargill.com
Shawn Starkenburg	THINKER	shawns@lanl.gov
SynBioSys	THINKER	synbiosys@icloud.com
Bryan	THINKER	btracy@whitedoglabs.com
katy	THINKER	kmchristiansen@lbl.gov
Jeffrey Dietrich	THINKER	jadietrich@lygos.com
as	THINKER	abshish@gmail.com
Abbott	THINKER	abbott@amyris.com
CRAIG	THINKER	craig.brown@nrel.gov
Michael F.	THINKER	mike.fero@teselagen.com
Siva	THINKER	siva.sivasubramanian@ee.doe.gov
pablocarb	THINKER	pablo.carbonell@manchester.ac.uk
Prasad Gupte	THINKER	prasad.gupte@ee.doe.gov
Steve V	THINKER	svandien@genomatica.com
Jay	THINKER	jdkeasling@lbl.gov
Stefan de Kok	THINKER	stefan@zymergen.com
John	THINKER	john.perkins@dsm.com
Michael	THINKER	michael.koepke@lanzatech.com
Dave	THINKER	David.anton@cellana.com
jodi.grgich	LEADER	jodi.grgich@inl.gov
Casey	THINKER	casey.lippmeier@dsm.com
RV	THINKER	rvance@nifa.usda.gov
valerie	THINKER	valerie.sarisky-reed@ee.doe.gov
rnatelson	THINKER	rnatelson@bcs-hq.com
David	THINKER	david.babson@ee.doe.gov
Adam Bratis	THINKER	adam.bratis@nrel.gov
Corinne	THINKER	corinne.drennan@pnnl.gov
Rafael Nieves	THINKER	rafael.nieves@ee.doe.gov
kgray	THINKER	kevin.gray@fhr.com
ianrowe	THINKER	ian.rowe@ee.doe.gov
Dave T.	THINKER	David.Thompson@inl.gov
Jay Fitzgerald	THINKER	jay.fitzgerald@ee.doe.gov
Nate	THINKER	nate@ginkgobioworks.com
Matt Lipscomb	THINKER	matt@dmcbio.com
Sarah Studer	THINKER	sarah.studer@ee.doe.gov
Nate	THINKER	nate@ginkgoboworks.com
Mehmet	THINKER	mcburdu@gmail.com

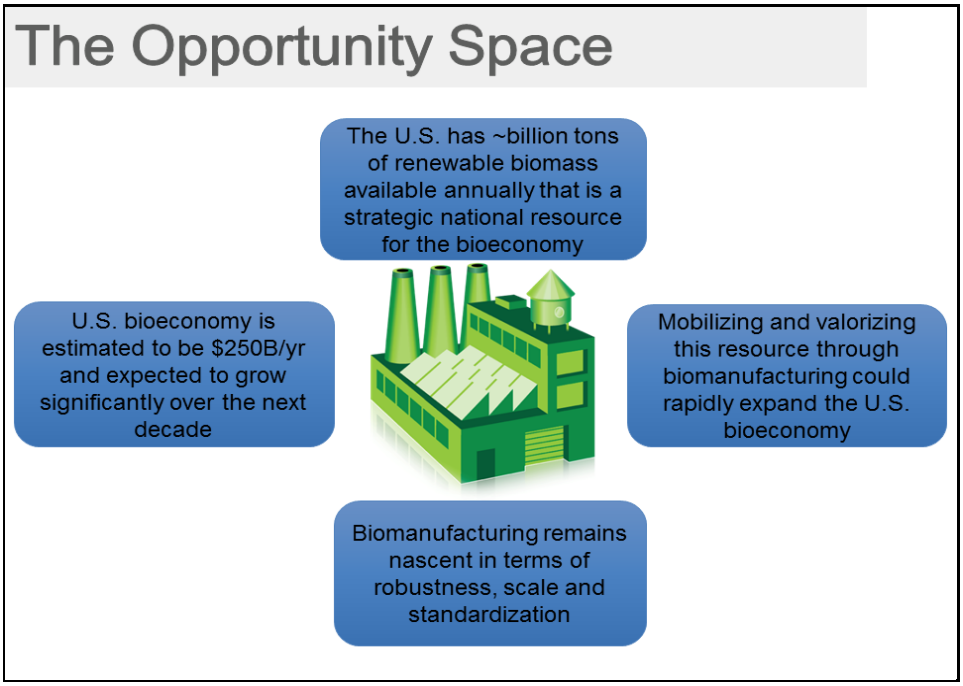
PRESENTATION: HOW WE GOT HERE





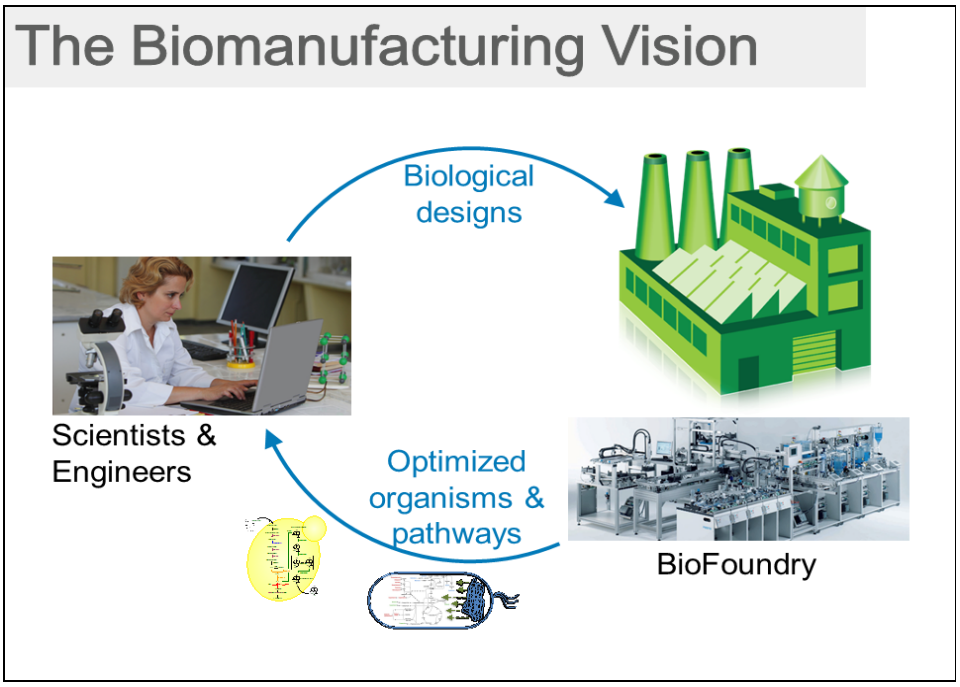
Comments

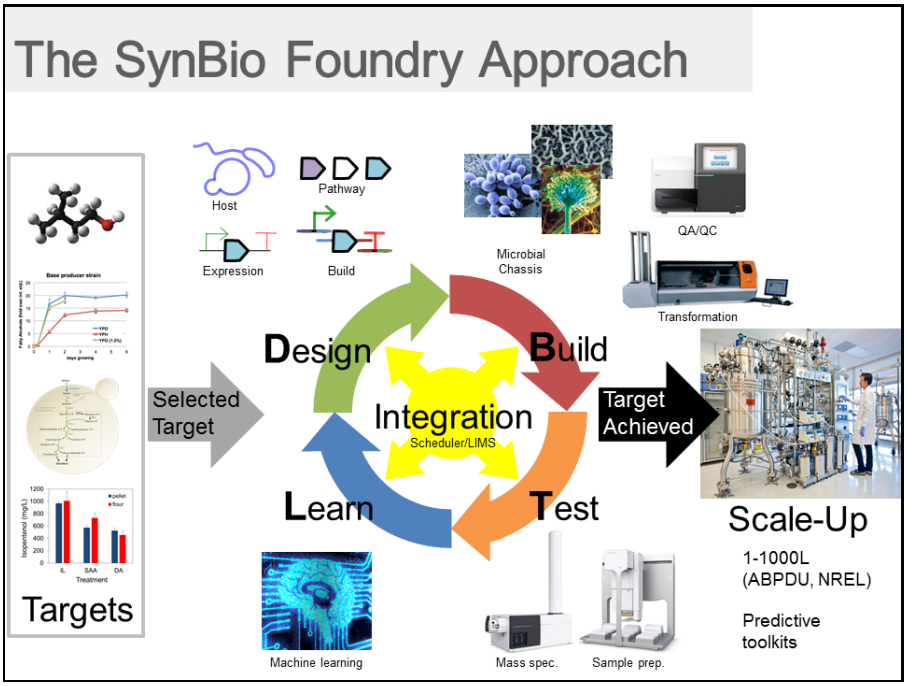
1. What is the basis for the reductions?
2. is there analysis to support these goal staements?
3. Where do the 40,60,40 numbers come from?
4. How will these targets be measured



Comments

1. Number for "grow significantly"
2. Ref for $250B/yr
3. The claim of 1 Billion tons per year "currently" existing is not accurate. This is a market scenario projection based on availability at specific price points, and in 2030.

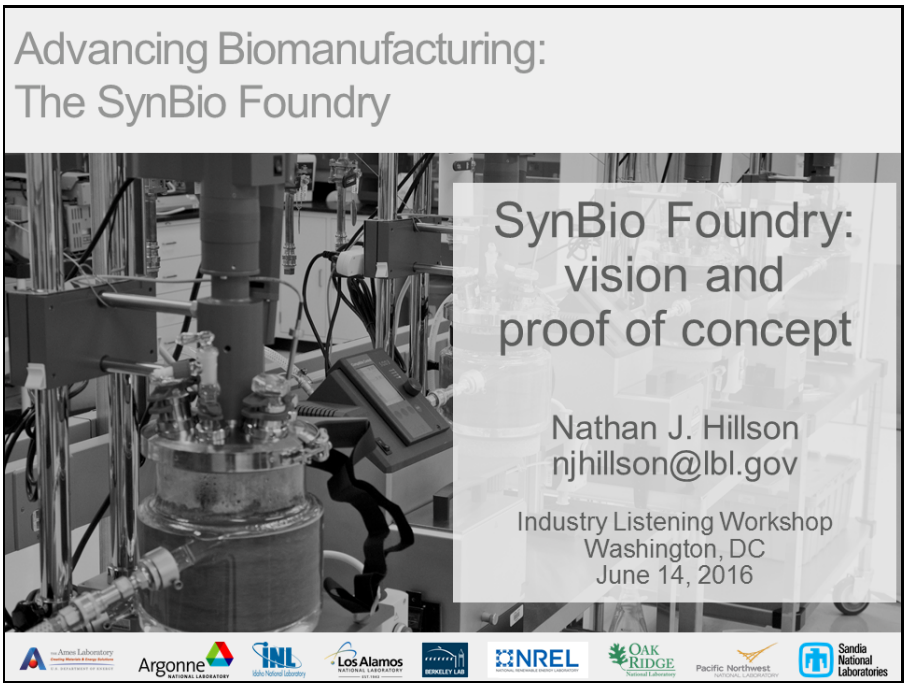


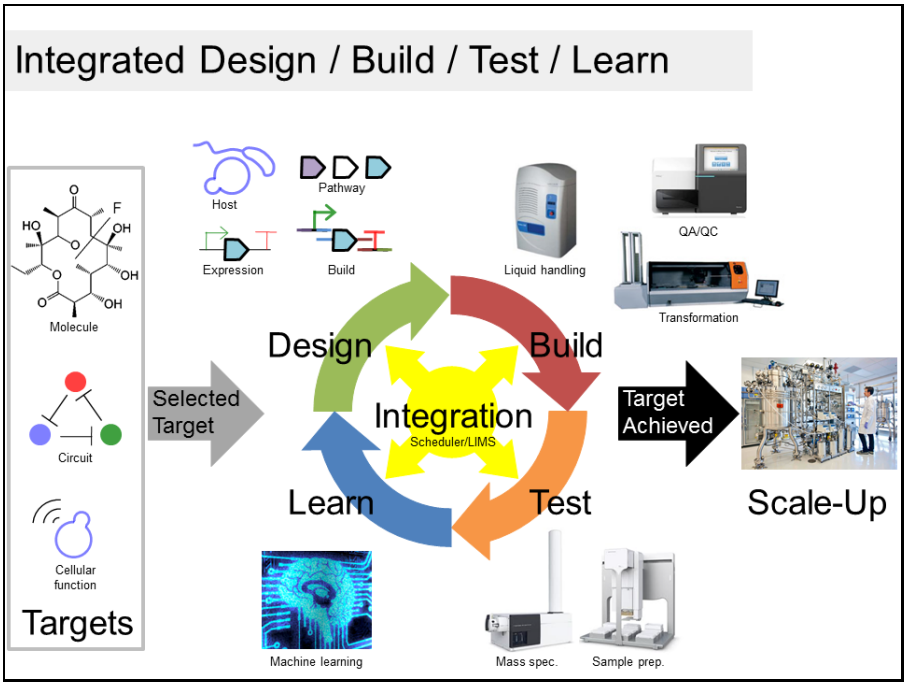


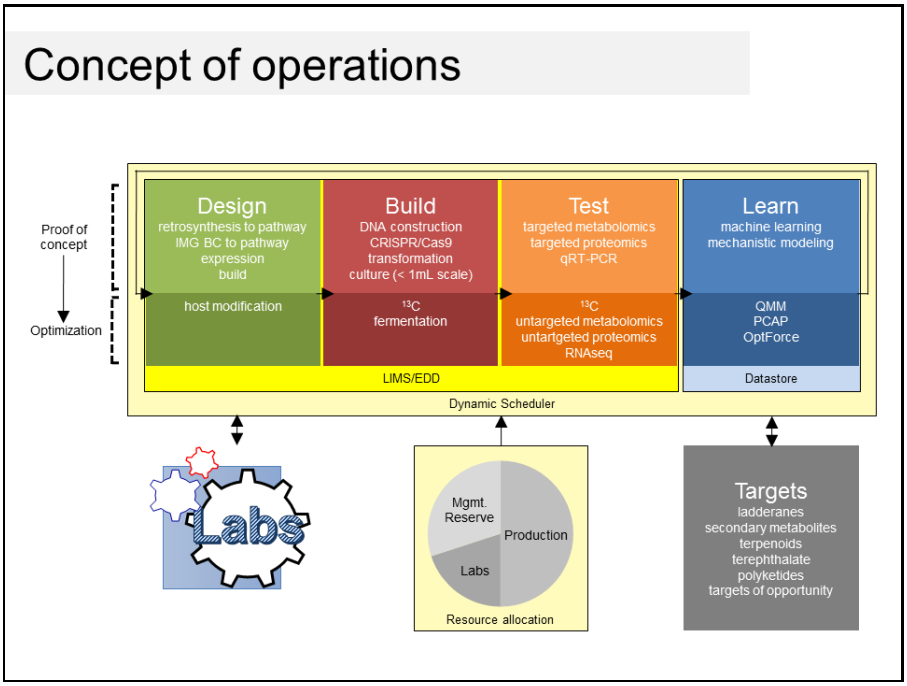
Comments

1. Industrial robustness is a requirement of a microbial chassis
2. What if a fundamental tool is needed but doesnt exist yet? How does this play into the foundry? Will it be developed under the foundry umbrella, or go to Office of science or NSF as a need?
3. How does seamless nature of integration work amongst far flung Natl. Labs with differing mandates?
4. Scale is dependent not just on what needs to be proven for the organism but also what has to be proven downstream

PRESENTATION: THE FOUNDRY PILOT

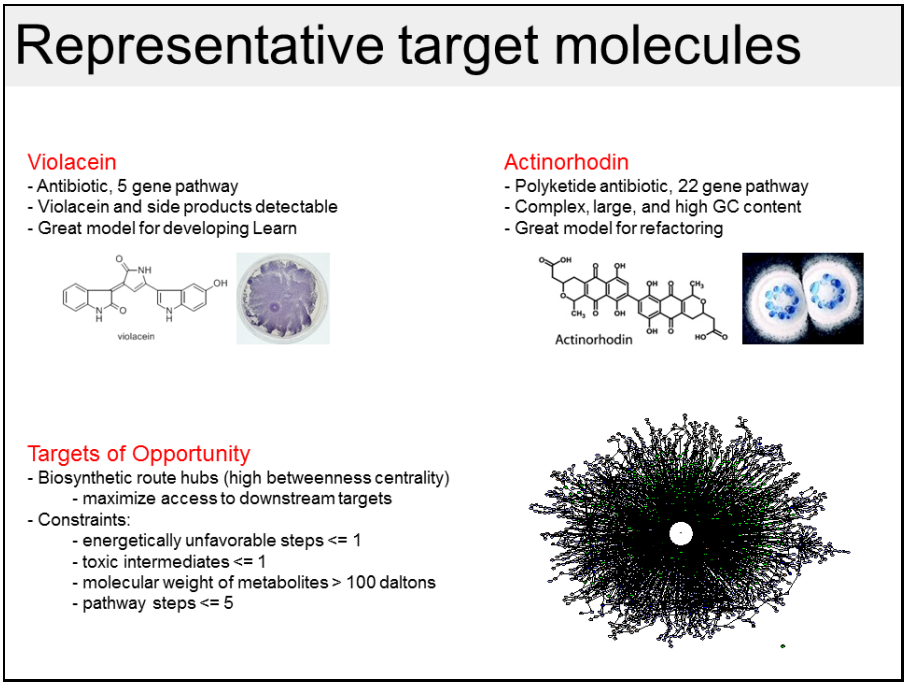






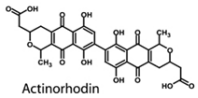
Comments

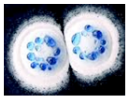
1. will there be a mechanism in place for new tools developed outside the foundry, to be onboarded into the foundry?
2. should be concerted effort to avoid Not Invented Here syndrome.



Actinorhodin

Actinorhodin: polyketide antibiotic produced by *Streptomyces coelicolor*, requiring 21 genes for biosynthesis. Cluster ~ 25 kb, %GC >70





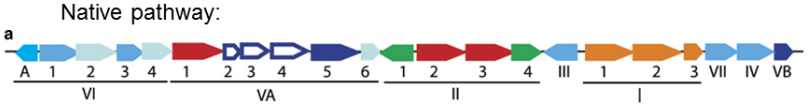
Why actinorhodin?

- Representative of many biosynthetic clusters (size, complexity, GC content)
- Pathway is well characterized (but never before refactored)
- Actinorhodin is readily detectable (blue/red pigment depending on pH)

Actinorhodin pathway refactoring

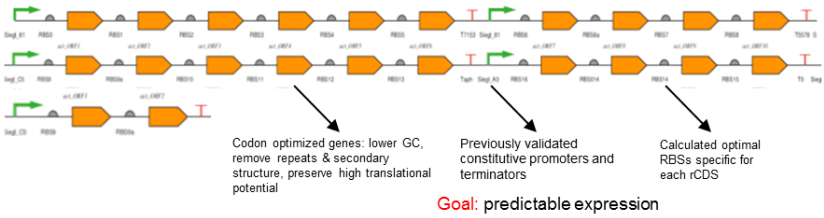
"Refactoring": Redesigning a complex set of operons that are highly regulated under native conditions to predictably achieve a phenotypic outcome under laboratory conditions

Native pathway:



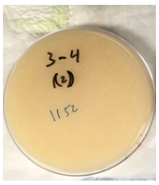
Highly regulated expression; unknown control system

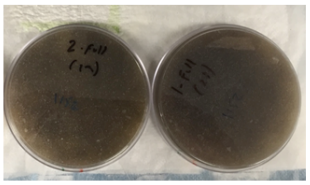
Refactored Design:



Goal: predictable expression

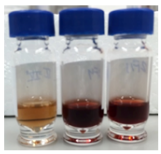
Actinorhodin strain results



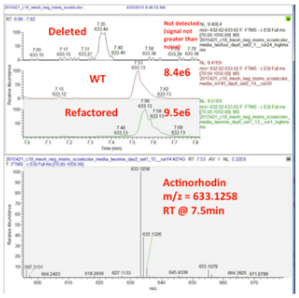


S. coelicolor Δ*act strain* (control)

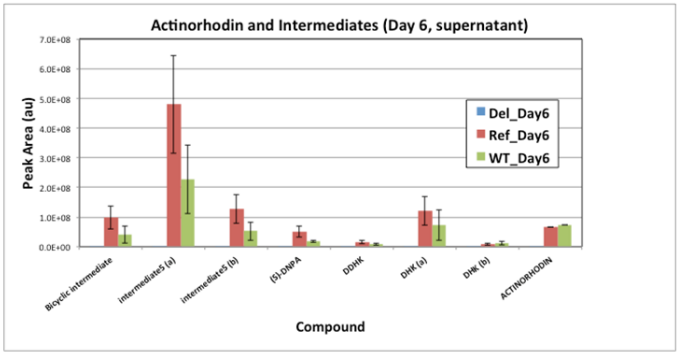
Refactored actinorhodin strains

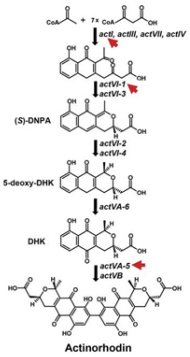


Extracts for LC-MS analysis



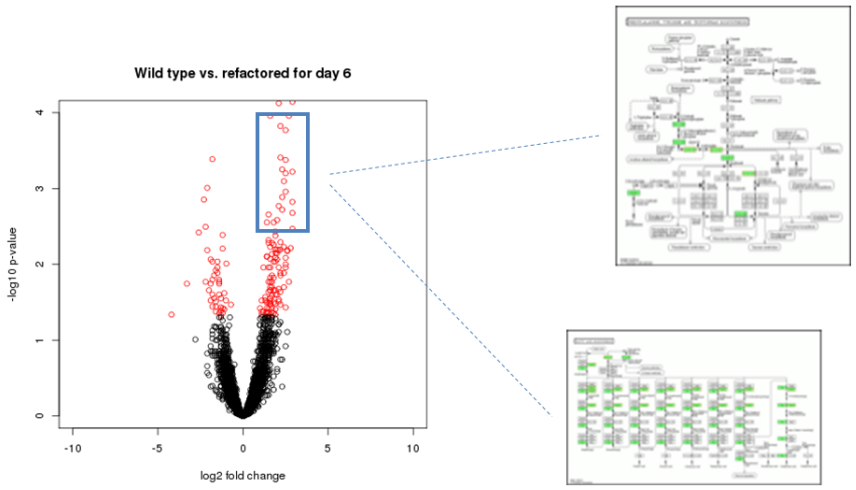
Pathway output is 100% of wildtype





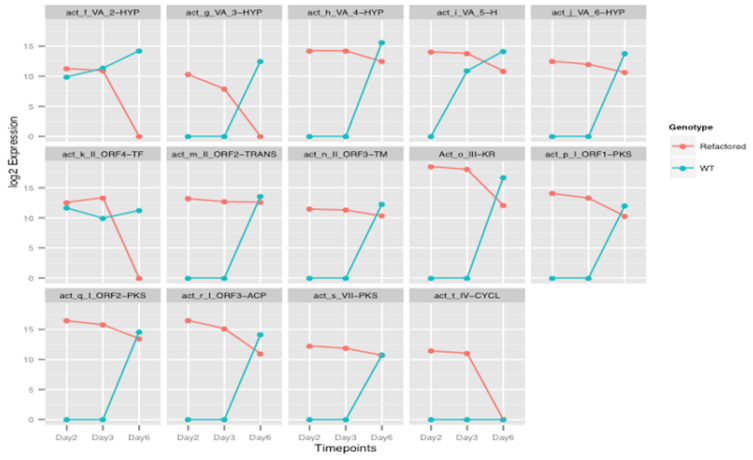
Constitutive actinorhodin production results in cell-stress response

Transcriptomics to detect and quantify over- and under-expressed genes



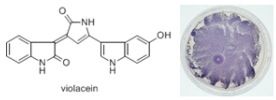
Constitutive actinorhodin pathway expression diminishes performance stability over time

Targeted proteomics to detect and quantify actinorhodin pathway enzymes

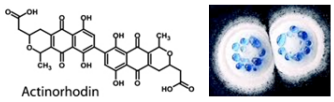


Representative target molecules

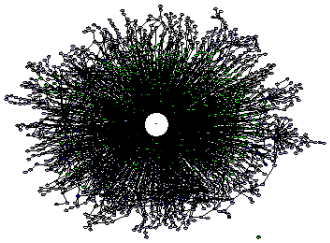
Violacein
- Antibiotic, 5 gene pathway
- Violacein and side products detectable
- Great model for developing Learn



Actinorhodin
- Polyketide antibiotic, 22 gene pathway
- Complex, large, and high GC content
- Great model for refactoring

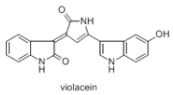


Targets of Opportunity
- Biosynthetic route hubs (high betweenness centrality)
  - maximize access to downstream targets
- Constraints:
  - energetically unfavorable steps <= 1
  - toxic intermediates <= 1
  - molecular weight of metabolites > 100 daltons
  - pathway steps <= 5



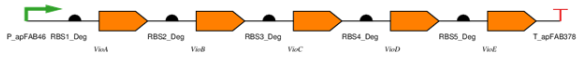
Violacein

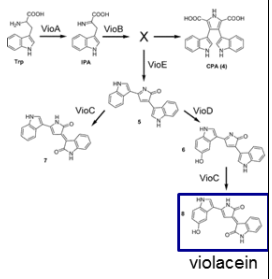
Violacein: natural antibiotic produced by *Chromobacterium violaceum*, requiring 5 genes for biosynthesis. Cluster 8 kb, %GC ~50



Goals:
- Generate large combinatorial combinatorial library
- Correlate molecule production to sequence and protein expression to learn critical features leading to optimized flux

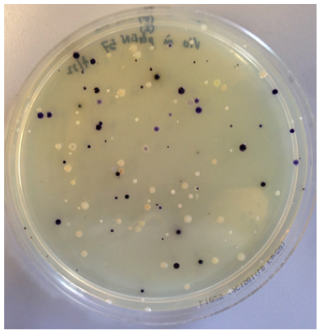
Violacein pathway design

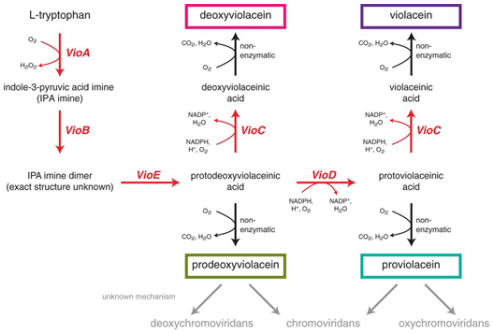




Design Features: Degenerate RBSs + multiple gene orthologs
50,000 strain variants

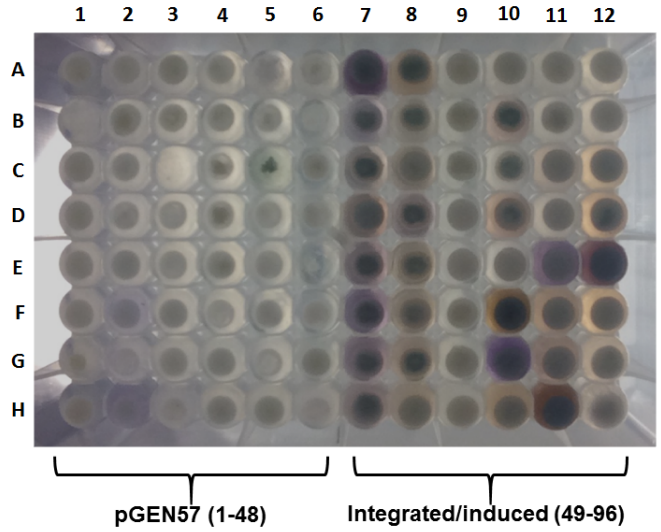
Violacein strain variants



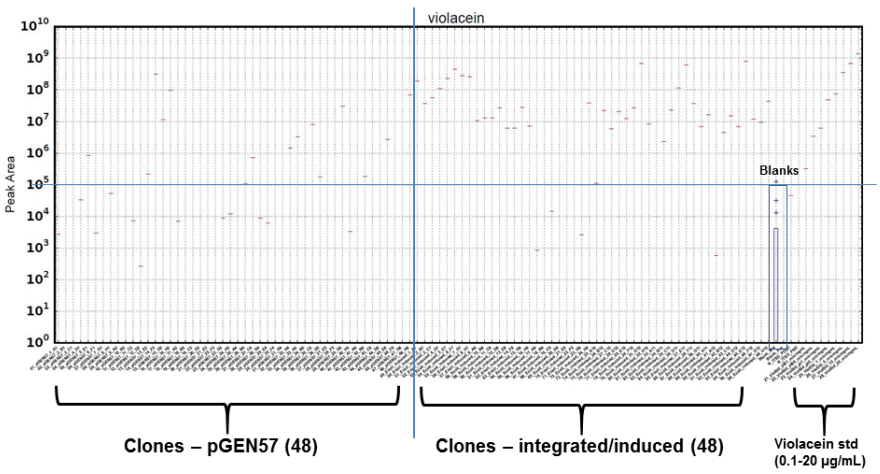


Plan:
Select 500 strains based on phenotypes.
Sequence, metabolomics and proteomics to build pathway optimization model.

96-well plate (individual clones)

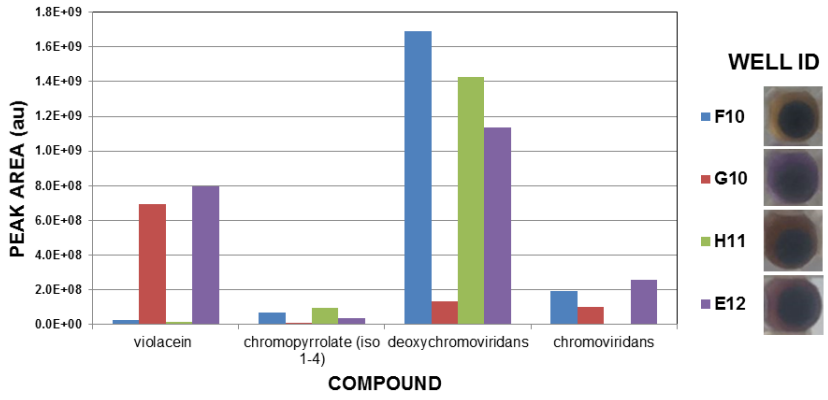


Violacein detected in each clone

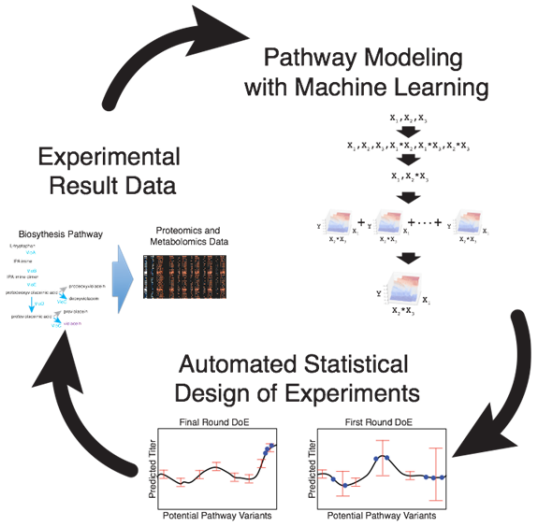


Select cut-off value for signal due to background / carryover from previous samples

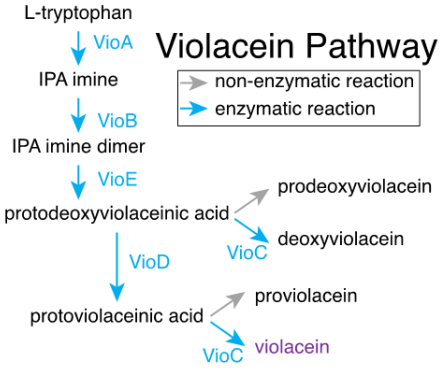
Relative amount of violacein and shunt products



Design of experiments and machine learning



Iterative improvement for violacein model



5 promoter levels for 5 Genes
= 5^5
= 3125 possible strains

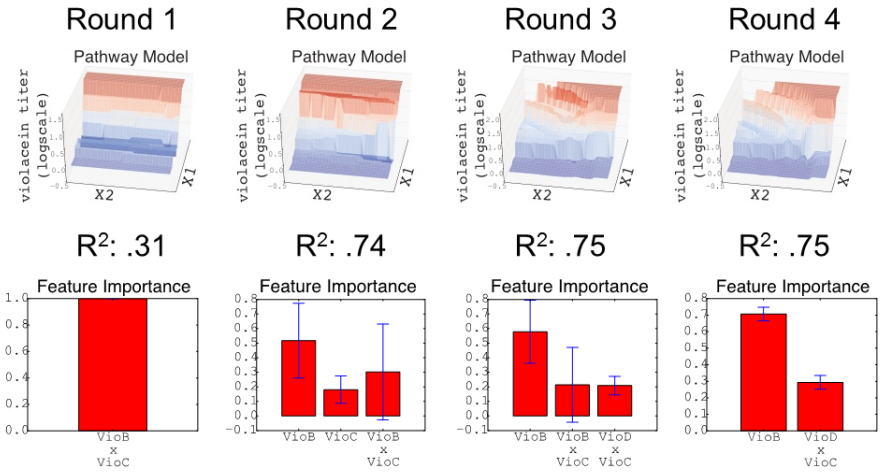
187 randomly selected strains build and tested

randomly divide strains into 5 sets

Data Source

Lee ME, Aswani A, Han AS, Tomlin CJ, Dueber JE. Expression-level optimization of a multi-enzyme pathway in the absence of a high-throughput assay. Nucleic Acids Research. 2013;41(22):10668-10678.

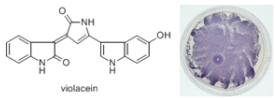
Iterative improvement for violacein model



Representative target molecules

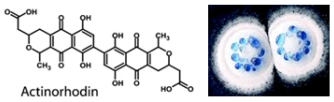
Violacein

- Antibiotic, 5 gene pathway
- Violacein and side products detectable
- Great model for developing Learn



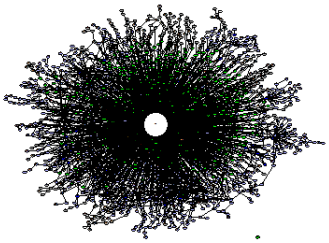
Actinorhodin

- Polyketide antibiotic, 22 gene pathway
- Complex, large, and high GC content
- Great model for refactoring

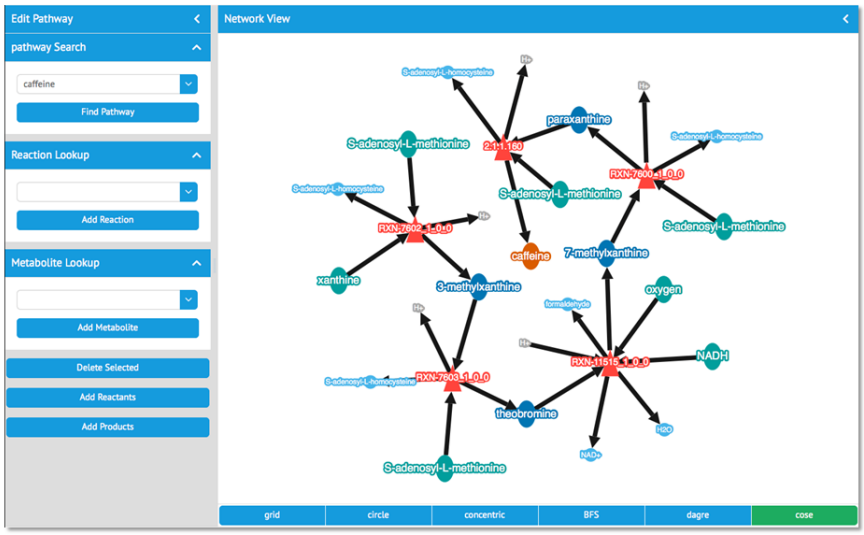


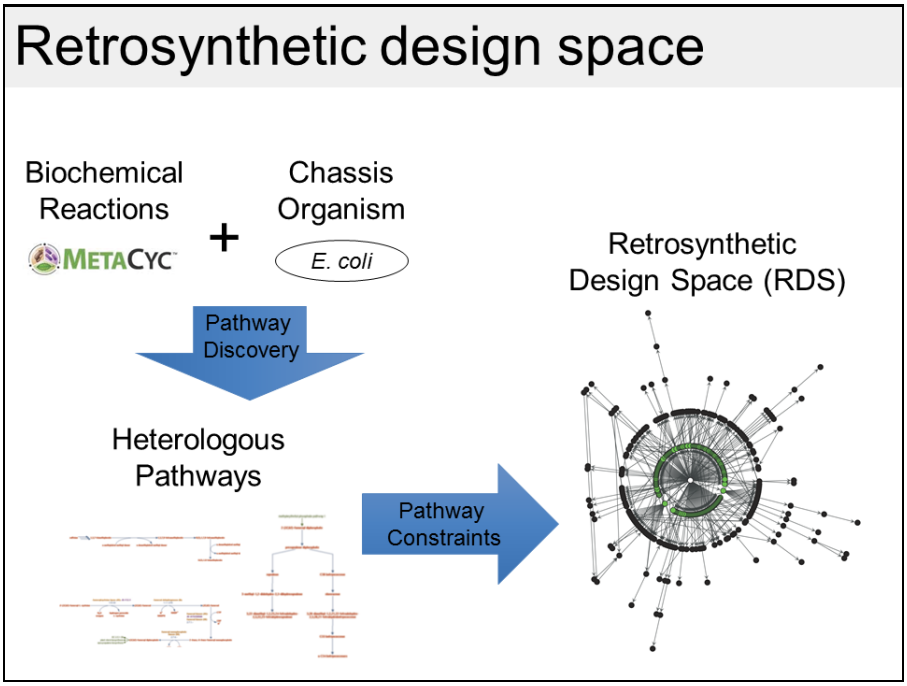
Targets of Opportunity

- Biosynthetic route hubs (high betweenness centrality)
  - maximize access to downstream targets
- Constraints:
  - energetically unfavorable steps <= 1
  - toxic intermediates <= 1
  - molecular weight of metabolites > 100 daltons
  - pathway steps <= 5



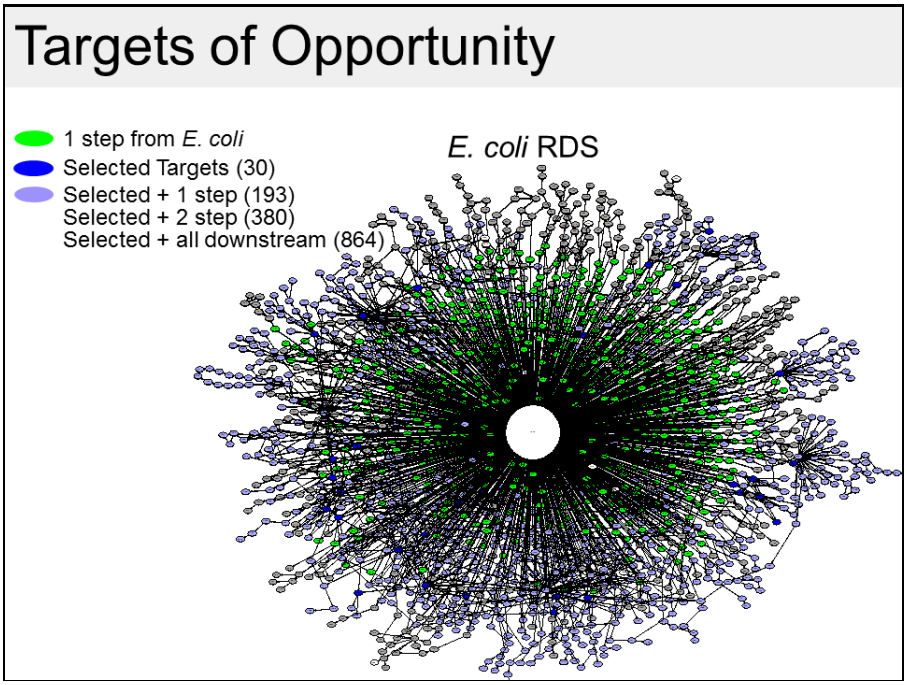
Retrosynthesis design





Comments

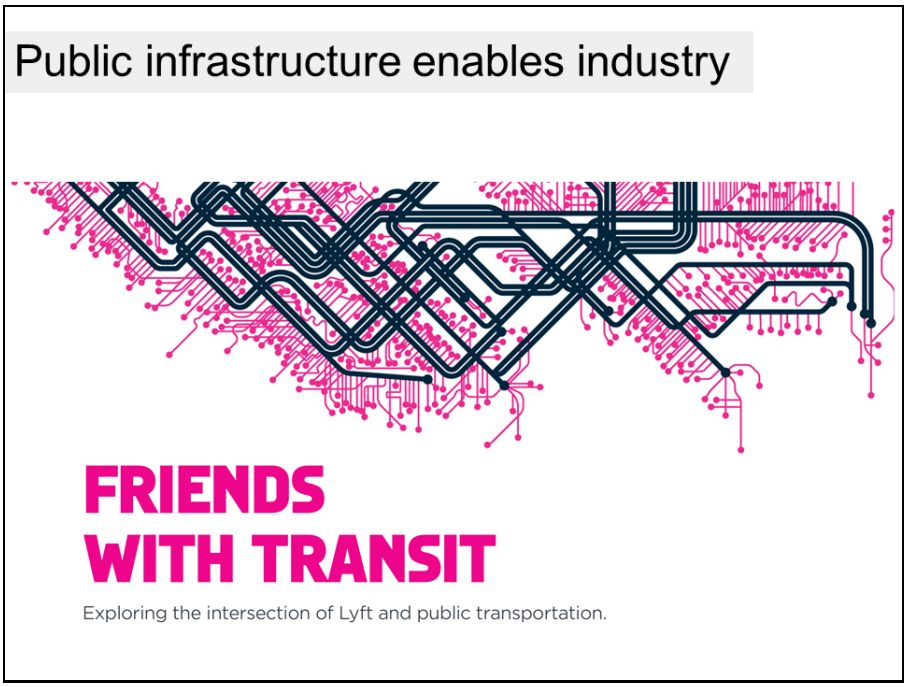
1. We shouldn't limit reaction space to those in MetaCyc or other databases. Consider promiscuous enzyme activities or the ability to engineer new substrate specificity.



Comments

1. The reuse/beachhead concept is powerful, and it's true that many companies will shy away from single target licensing strategies.

2. super secret proprietary molecules dont really exist, as there are key molecules that multiple companies will want to go after, so it may be difficult to develop on a common similar molecule, and then send the company off with the common knowledge...once that common piece exists, someone else can come in and use it for possibly same end molecule without any investment in the common part. Setting up a race to the final molecule and who can patent it.

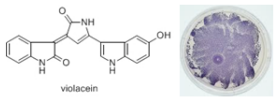


Comments

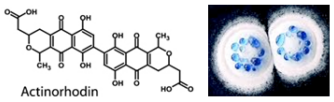
1. Very relatable analogy. Can it be made to work? This is probably key to the entire proposal (see comments on previous slide) and goes to the heart of the public/private partnership.

Summary of Progress to Date

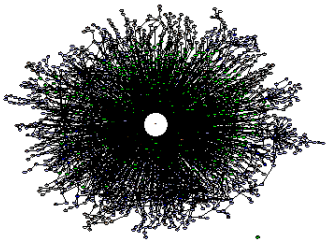
Violacein
- Antibiotic, 5 gene pathway
- Violacein and side products detectable
- Great model for developing Learn



Actinorhodin
- Polyketide antibiotic, 22 gene pathway
- Complex, large, and high GC content
- Great model for refactoring



Targets of Opportunity
- Biosynthetic route hubs (high betweenness centrality)
  - maximize access to downstream targets
- Constraints:
  - energetically unfavorable steps <= 1
  - toxic intermediates <= 1
  - molecular weight of metabolites > 100 daltons
  - pathway steps <= 5

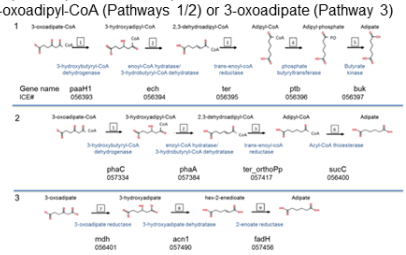


2.5.3.104 Agile Biomanufacturing – Adipic Acid Production in *P. putida* and *S. venezuelae*

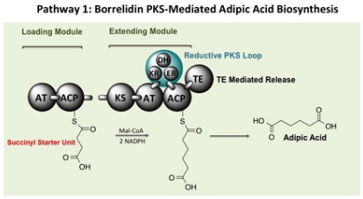
Project Objective

- Rapidly on-board new hosts and improve molecule production titers.
- > 100-250 mg/L adipic acid by FY16 Q4
- > 1-10 g/L adipic acid or precursor by FY17 Q2
- Precursor to a larger Foundry effort to address biomanufacturing challenges through advanced design, build, test, learn cycles leveraging synthetic biology and predictable scale-up.

Adipic acid production in *P. putida* from 3-oxoadipyl-CoA (Pathways 1/2) or 3-oxoadipate (Pathway 3)

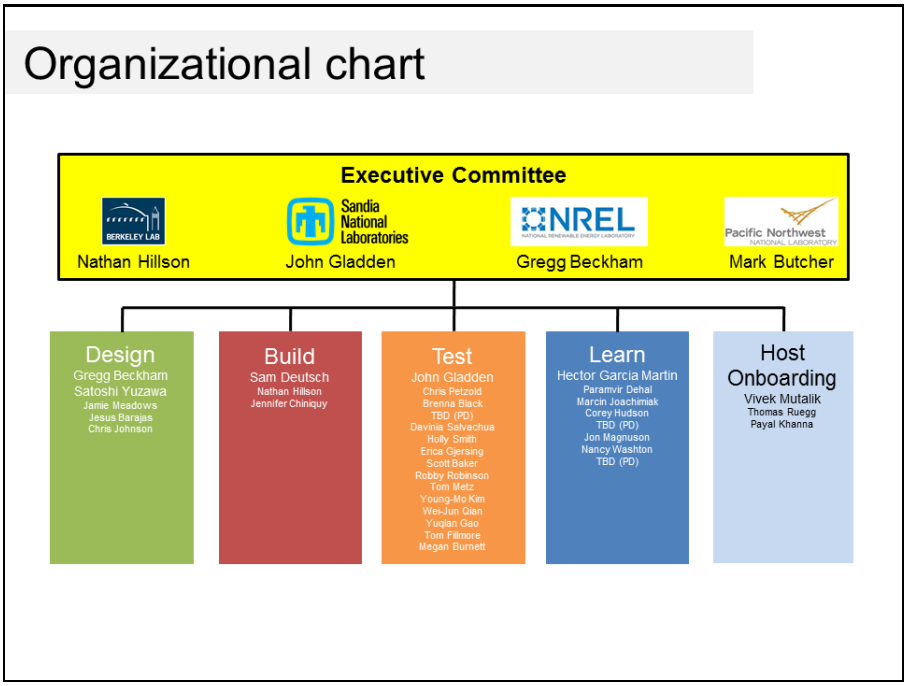


Engineered PKS system to produce adipic acid in *S. venezuelae*



Comments

1. adipic acid or the salt? very different processes
2. Also should have yield and productivity targets

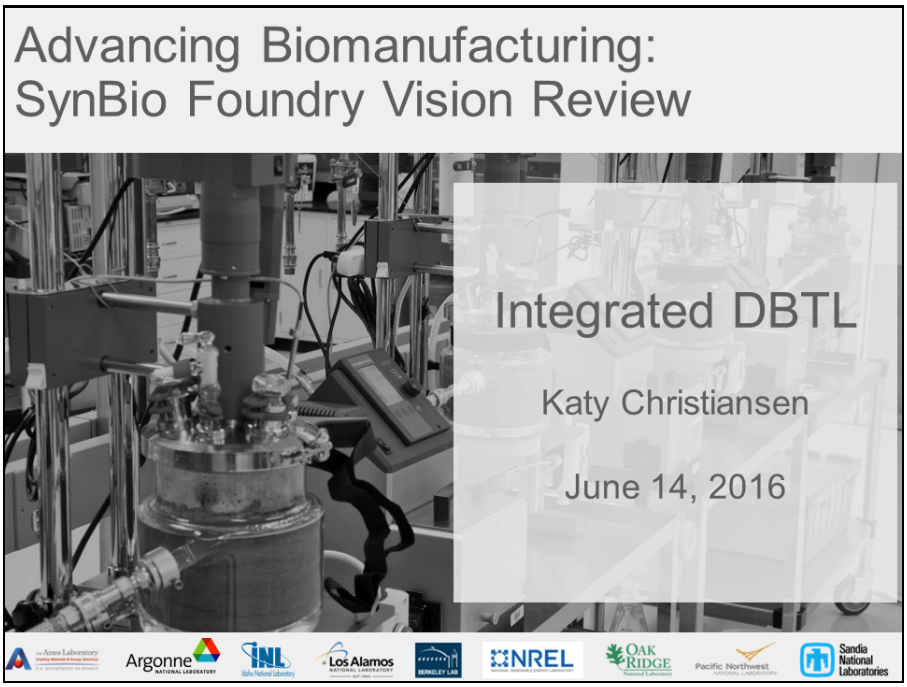


Comments

1. Someone asked earlier how this effort is different from Synberc ERBC? Do we want to discuss that?

1.

2. PRESENTATION: INTEGRATED DBTL



Integrated DBTL

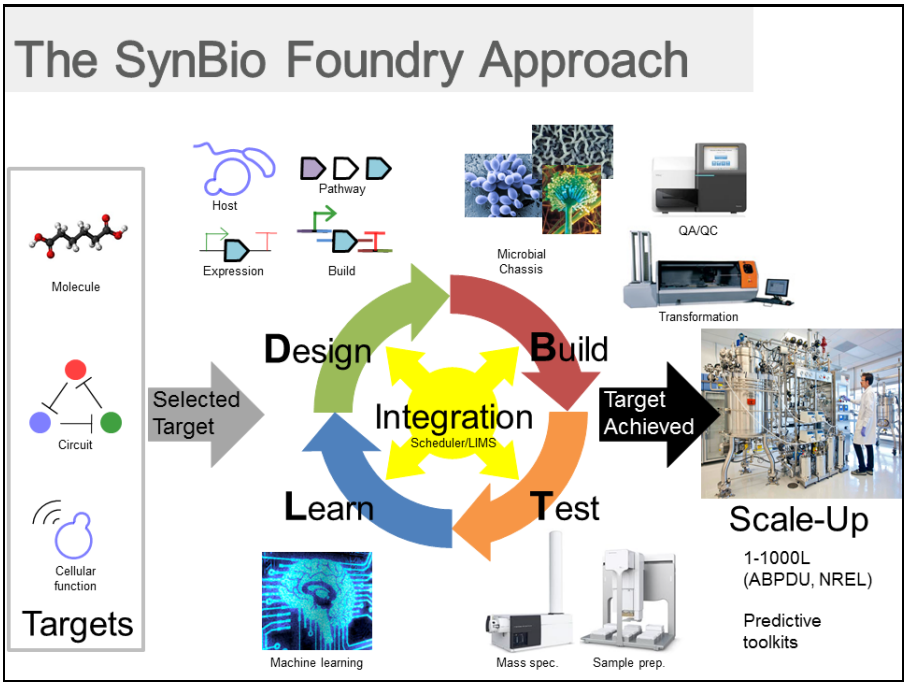
Goal: Integrate tools, technologies, and overall process analysis into a platform to enable more efficient biological engineering

Approach:

- Integrate software, databases, instruments, and other tools for bioprocess design with machine learning and statistical modeling
- Consider process conditions, host organism, technoeconomic analysis, and life cycle assessment as parameters that influence design
- Subject matter experts embedded in DBTL/I team for hosts, analysis, and scaling to ensure that the DBTL platform overcomes critical barriers for efficient biological engineering

Metrics for Success (within five years):

- Reduced cycle times for DBTL (9 months to <3 months)
- Increased capacity for host/target combinations (from 5 to >100)
- Increased number of hosts that work with platform (from 5 to >20)

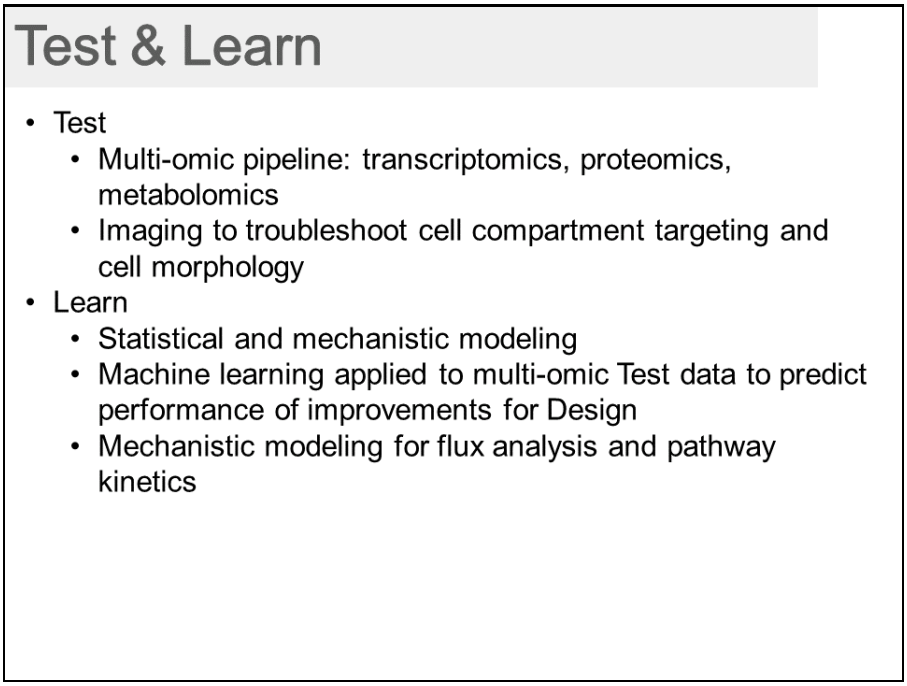


Design & Build

- Design
  - Integrated toolchain software architecture with push notifications
  - Biological pathway design that pulls from ”parts” and experiment databases
  - Process considerations including host organism and downstream separations and upgrading incorporated in Design
  - Technoeconomic analysis and life cycle assessments incorporated into decision making
  - Hands-on contributions from subject matter experts to codify knowledge into software
- Build
  - DNA construction and transformation into desired host
  - Produce material for test
  - Forward genetic screens developed by on-boarding team

Comments

1. Will LCA include indirect land use?
2. Entry points may be at different levels, e.g. starting at target molecule with retrobiosynthesis or having already pathway or host. Standardization of requirements is critical to manage this. Some of this may be stage-gated (e.g. retrobiosynthesis)



Comments

1. The Build to Test handoff is critical for proper sampling handling, data quality etc. to make sure that data is worth looking at.
2. Build owning culture creation can be risky across multiple sites.
3. For Test, make sure to have sufficient capacity for routine analytical measurements (product titer, byproducts) to match capacity of high-throughput strain construction and culturing
4. Quality control on data- do you have good carbon balance? Is the result reproducible?
5. Consider evolution to start with a better chassis strain for further cycles.
6. QC important to make sure data is relevant as as expected to make Learn part of cycles relevant and not drawing wrong conclusions.

Integrating DBTL

- Sample tracking across distributed consortium
- Connect databases through API
- Automate dynamic resource allocation through a system scheduler

Comments

1. will AOP allow for funds to move to labs in a dynamic fashion? What control will DOE have? Will there be definitive milestones that wont change regularly in this dynamic state?
2. Dynamic resource allocation through a systems scheduler? Across the labs? This sounds tricky.

Implementation

- Crops
  - Target/host combinations will be grouped into "crops" based on risk, metabolic pathway, and analysis
  - Initial crop will be low-risk to establish the SBF
  - Later crops will focus on high-impact targets and may include "targets of opportunity"

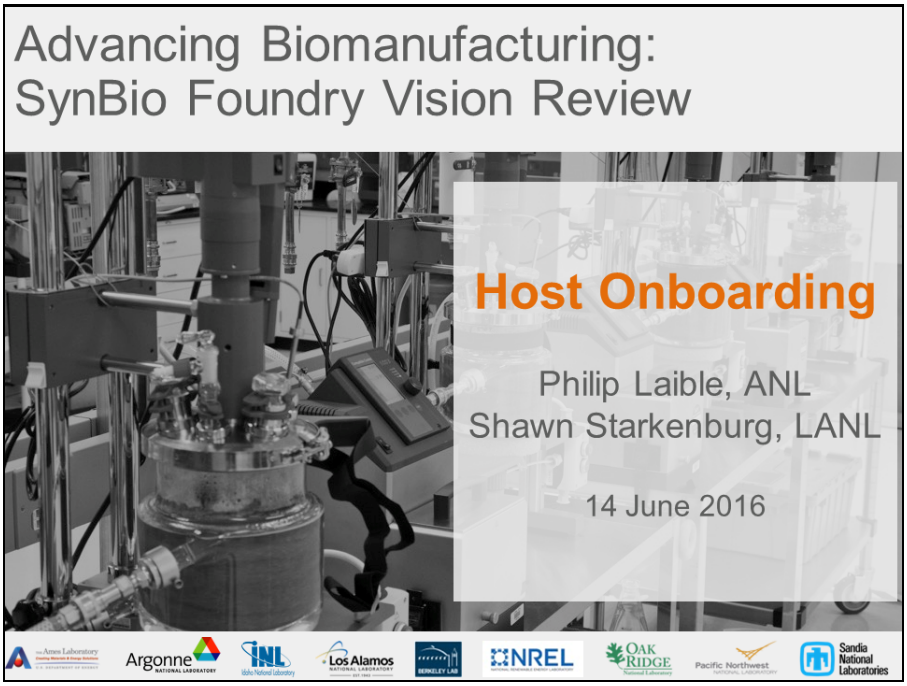
- Metrics

	FY17	FY18	FY19	5 years
Number of SBF hosts in operation	5	7	10	>20
Number of concurrent target/host combinations per year	5	15	30	>100
DBTL cycle time	9 months	8 months	6 months	<3 months
Table 1: Cycle time and capacity targets for the SBF Strain samples analyzed per year	35,000	50,000	75,000	>100,000

Comments

1. Will target/host combination be only Proof of Concept? Suggest the Foundry try improve established production

target/host combinations
2. Suggest mixing in low-risk and high-impact targets from the outset. National labs should be going for high-impact high-risk.
3. early hosts will likely have the most information and tools available for them. Later ones not so much. How will the dramatic decrease in cycle time be effected by this
4. Consider possibility of partial DBTL cycles or multiple cycles in progress concurrently.
5. Partial DBTL cycles are quite common for us at Ginkgo.
6. might be useful to have a quality control guide, or set of processes that users follow to send samples, along with validation of quality process built in at intervals to be able to accommodate the multiple labs involved and reduce errors
7. Check numbers in final column. Strain samples per cycle goes down. Should be more like > 40-50k/cycle? Also, much of flux through workflow depends on decreasing cycle time. How much is due to parallelism and automation and how much to working faster in series?
8. Also suggest the Foundry try to improve targets from low C-flux pathways
9. will groups like Office of science and NSF be cranking out hosts and tools to continue to populate the needs of the synbio foundry? Will synbio foundry learn something in early cycles that will inform OS and NSF.? Should find a way to ensure that there is collaboration with these groups



Comments

1. What does it take to get a host accepted by industry? Get some information out at some level of scale, but you still won't know about minor reactions and other complications that dont
2. Will find that there will be very few tier 4; more like 0 or 1
3. One criteria that is missing: regulatory environment for host and likely disposition for that host
4. What makes a organism industrially relevant is that it's been used in industry before- should be one of the criteria; most of the criteria are just a matter of work. Would recommend looking at what's been used in industry as a starting point
5. How do we get at those hosts? A lot of literature on non-proprietary hosts, or get ahold of deposited industrial hosts
6. Innovation over optimization: can we do both? Even for small companies, it's important
7. Need to figure out how to prioritize- is optimization more useful for more than one person/company?
8. How much would you develop a host? What about hosts that may have no genetic tools? May need to find new organisms though other mechanisms- translate once utility is known
9. Feedstock criteria is important- use low cost or waste feedstocks
   9.1. Use glycerol waste from first gen ethanol; cleaner than hydrolysates
   9.2. Processes that use CO2 or methane
10. Mesophilic vs. thermophilic is another criteria
11. Capabilities that are not accessible with E. coli or yeast
12. What would make it worth it to industry to use a new organism? That's a hard question- have to evaluate risk of use for (presumably) new process
13. E.g. Adipic acid will only work at a low enough pH that it's protonated- think about operating conditions to purify and separate process downstream; thermophilic might make sense since you could volatilize the product
   13.1. Need to start with separation and then figure out what host is
14. How to use anaerobic hosts and do high-throughput screening? Need to think about how that would happen
   14.1. 5-10 ml but would need to narrow down number for screening
15. Issue of looking at targets- class targets and select organisms for classes of targets

15.1. A lot of literature- look at Top 10. Driven by what people knew what the metabolic pathways could do
15.2. Don't need to know specific targets; could look at what targets have been publicly disclosed. E.g. organic acids
16. Tools for existing hosts?
16.1. Why wasn't bacillus on the list?
16.2. heterotrophic production for algae; for photosynthetic organisms, increasing photosynthetic yield would have bigger bang for the buck; algae is still untapped area here
17. Need to be able to tolerate contamination- process will be dirty
18. Could be a refinement after selection: high flux through certain metabolic nodes
19. Look at reaction mechanism to see if they are covered
19.1. If co-factors are required, etc.
20. Regulatory hurdle- things like staph would be too high to ever pursue
20.1. What are the traits from those organisms that are useful- can they be transferred or can they be found elsewhere?
21. How far do the labs need to take the work?
21.1. Advantaged or possible where is wasn't possible
21.2. Think about how much risk was minimized by partner
21.2.1. Things don't start looking weird until 100-200L; can't see until you're there. But if that was done and understood, that would be valuable to industry; small scale is not predictive of downstream issues
21.2.2. Can mitigate heterogeneity, mixing time, etc. at smaller scale; tolerance to heterogeneity would helpful for platform hosts
21.3. Licensing a new organism? Make very low cost we can- essentially a public good for public money- is that reasonable?
21.3.1. Have licensed expression system for very little money- cost is much less than cost of product made
21.3.2. Probably depends on strain; but Cargill example is good: proved that it works in many different processes and people wanted to use
21.3.3. Middle ground: important to partner with industry, first group gets partner on it for licensing; but really need to get into specifics to figure out relationship
21.3.4. Want de-risked to certain level- lab could demonstrate to lactic acid and industry could take for its own interest since it works for product X
21.3.5. Is exclusive license for a certain period of time a way of managing it?
21.3.6. What could the SBF do to use a new organism? How can we minimize "cost?"
21.3.6.1. Phage contamination- track record in large scale fermentation; has it been used in industry and does it work?
21.4. Toolbox (may be organism specific): plasmids and chromosomal work

Goal: Evaluate, develop, and onboard a comprehensive pool of industrially relevant hosts into the DBTL platform

Need/Justification

- Industry stakeholders have indicated a need for organisms representing a broad range of metabolic space and manufacturing potential, at scale

Approach & Timeline

- Host selection driven by target molecules & desired traits
- Informed via TEA, LCA + industry/academic/government input
- Establish and standardize criteria for onboarding
  - Develop a tiered system of characterizing/manipulating host 'functionality'
  - Perform R&D to 'upgrade' tier status of candidate hosts
- Onboard at least 15 new organisms by year 4

Metrics For Success

- Time savings for adoption of new hosts by the biomanufacturing industry
- 'Graduation' of new hosts to advanced tiers for process flexibility
- Integration of new hosts into SynBio Foundry

Host Inventory & Selection

INITIAL HOSTS

1. *Streptomyces venzuelae*
2. *Pseudomonas putida*
3.
4. **TBD in Year 1**
5.

Proteobacteria
Pseudomonas
Zymomonas
Actinobacillus
Methylomicrobium
Gluconobacter
Enterobacter
Burkholderia
Methylobacterium
Bradyrhizobium

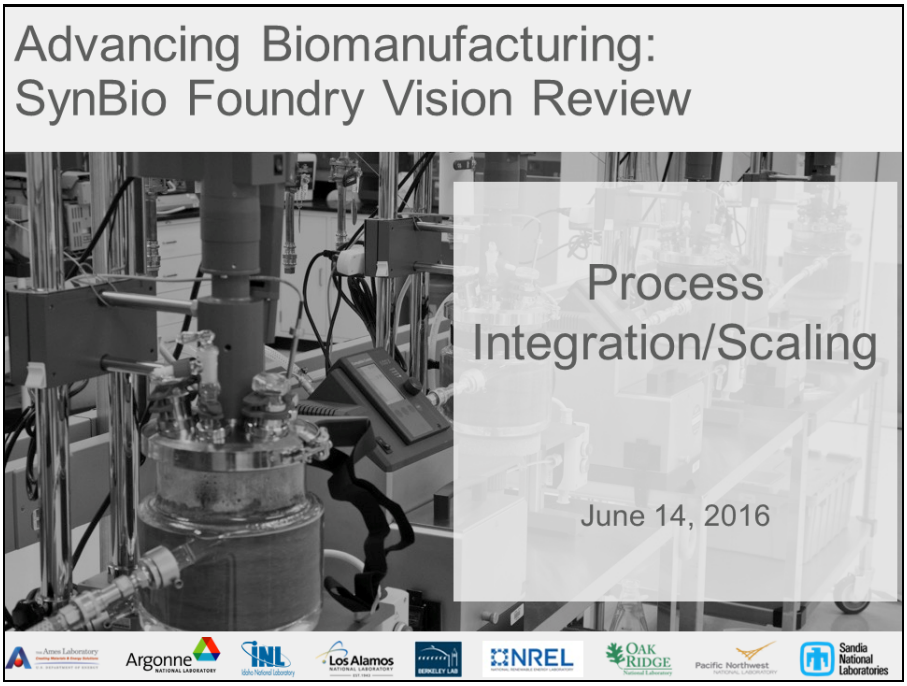
Actinobacteria
Corynebacterium
Rhodococcus
Streptomyces

Yeasts
Trichoderma
Rhodospordium
Lipomyces
Aspergillus
Pichia

Firmicutes
Clostridium
Thermoanaerobacterium
Caldicellulosiruptor
Moorella
Alicyclobacillus
Geobacillus
Staphylococcus

Algae and Cyanobacteria
Synechococcus
Synechocystis
Chlamydomonas
Picochlorum
Nannochloropsis
Chlorella

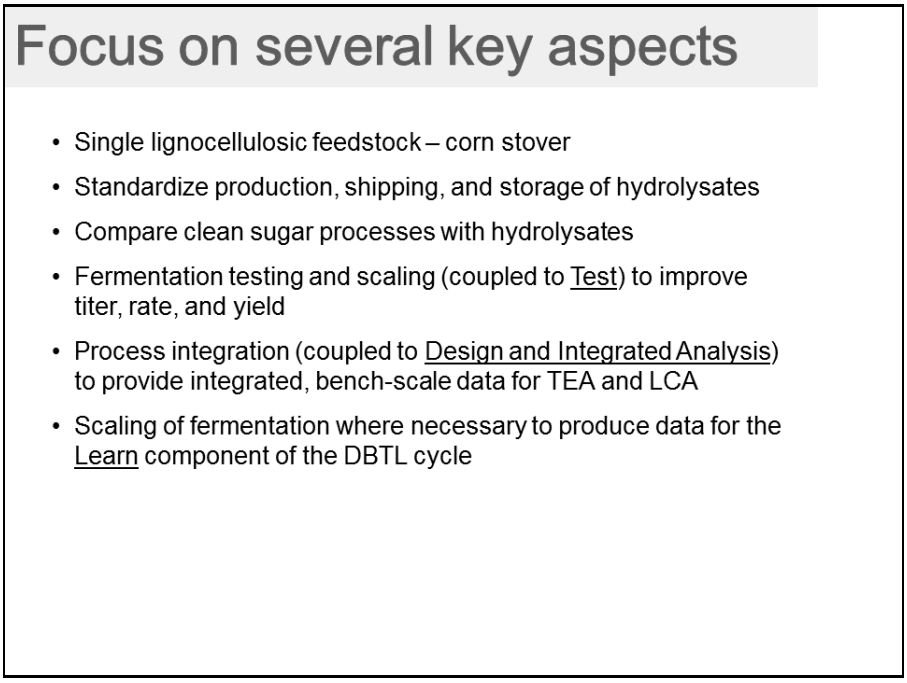
Archaea
Methanosarcina
Haloferax



Process Integration/Scaling

A primary output of the DBTL cycle, as well as a major component of Design and Test components of DBTL, involves process integration and scaling for the selected target molecules in the proposed SBF effort

- **Critical for understanding strain performance**
  - In the context of an overall bioprocess
  - Inform translation to an industrial setting
- **Strategy**
  - Consider relevant feedstocks beyond clean sugars
  - Cell culture at increasing scale
  - Identify inhibitors and toxins inherent to the feedstock as well as produced during pretreatment
  - Guide the development of downstream processing steps
    - Product separation
    - Purification
    - Upgrading
  - Validate achievable titer, rate and yield
  - Provide data for analysis activities



Comments

1. For any given project, use the same hydrolysate and methods of standardization (tracking batches)
2. Consider other feedstocks at some point?
3. Should also consider other "cheap" feedstocks, e.g. glycerol syrups from 1st gen ethanol process, less refined C-sources from from sugar beet and starch hydrolysis processes
4. Why default to corn stover?
   - 4.1. Historic association with ethanol
   - 4.2. Need to use a feedstock that is nationally available
   - 4.3. Wood as a consideration??
5. Very difficult to hear the speaker
6. Consider gaseous feedstocks (syngas, CO_2)

Approach

- Leverage currently funded efforts in feedstock handling and pre-processing to provide uniform corn stover compositions
- Leverage advances from other DOE-funded efforts for improved feedstock properties as developed
- Produce and ship hydrolysate to SBF partners in 1-100 L quantities as needed for the Test component of DBTL, as well as for initial screening of candidate hosts
- Fermentation testing
  - As needed when titer, rate, and yield measurements need to move beyond results obtainable in shake flask testing experiments
  - Most fermentation testing will be done at either small scale (e.g., in µ-scale multiplexed bioreactors) up to the 500 mL scale
  - Titer, rate, and yield will be the primary objective

Approach

- Fermentation optimization
  - Crucial for both the Test and Learn components of the DBTL cycle
  - Conducted on promising strains identified in shake flask trials
  - When titer, rate, and yield improvements can be gained by moving to controlled bioreactors
  - 'Omics measurements will be employed for bioreactor tests as well to identify metabolic bottlenecks and to inform Learn.
- Scaling will occur beyond 500 mL (up to 300 L) where needed for harvesting larger biomass samples
  - Transcriptomics experiments
  - Cases where the Learn component of the DBTL cycle would benefit from scaling up (or down) for predictive scaling purposes
  - When titer, rate, and yield targets are reached at smaller scale
  - When larger-scale production of a target molecule is needed for demonstration purposes

Comments

1. Correlation of titers from MTP to fermentation (fed-batch) should be emphasized.

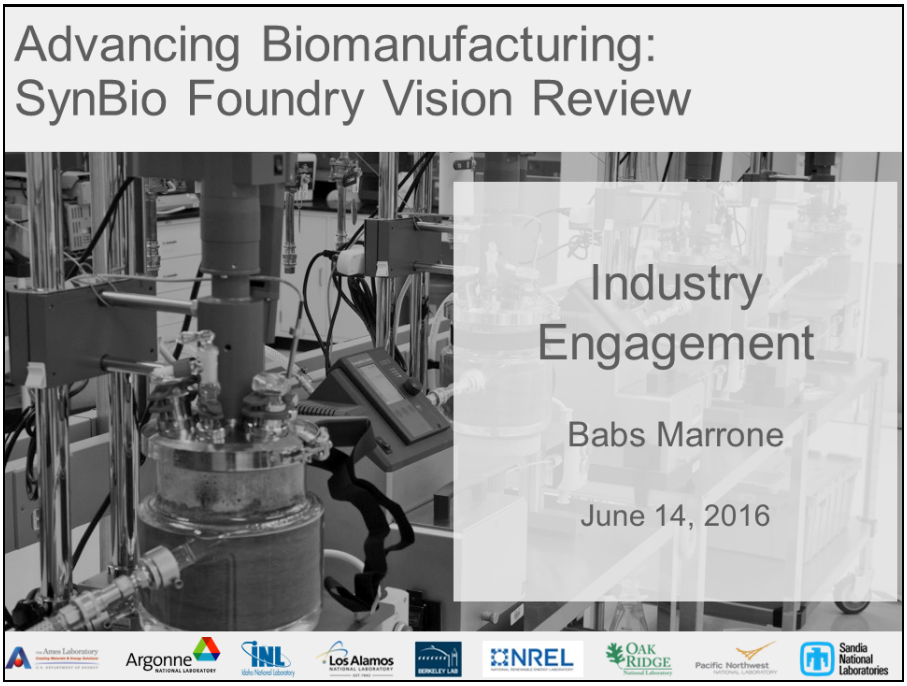
Discussion Questions

- What works about the proposed plan?
- What doesn't work?
- What should be improved?
- Has a critical consideration been missed?
- How will this be impactful over business as usual?

Comments

1. What does work?
   1.1. INL's extensive database and pedigree
2. Managing feedstock variability
3. Any reason to consider a secondary standardized feedstock?
4. What about using 3rd party feedstocks?
   4.1. "startup" feedstocks may still vary over time; Renmatix like processes should not be considered standardized yet; so in that case, NREL feedstocks may be best starting point
5. Transfer to feedstocks that are more likely to be adopted (waste, gaseous, cardboard); need to evaluate how robust a process is against multiple feedstocks
6. Recycables are a restriction for feedstock
7. Will base strains for organisms be tested against these common hydrolysates? (Yes)
8. Consider a secondary, standardized (real world) feedstock? Think about hot water-pretreated wood
   8.1. Agreed that project needs a second, real-world feedstock with an SOP
   8.2. Could be added as part of host screening process
9. Maybe just need to think about getting to a starting point, so a clean feedstock might be ok, but would be better to understand performance on different feedstock
10. Need to bake in coordination with separations consortium
11. Need to make sure we don't lose sight of focus on downstream processing
12. Not just inhibitors, but everything else that's in there and what happens later is key
13. need to consider about what other feedstocks will have more favorable quality parameters of downstream work
14. Would be good to have a strong relationship with a few companies that would work with us on a downstream process.
15. Where do you plunk down what SynBio is doing? What is in scope?
16. What is the minimum number of questions that need to be answered: It depends!
17. Thinking of it as technology push, but that's very difficult. Needs to be more of a partnership with industry to get more of a market pull

18. thoughts on getting real market pull data from industry- hold an organism selection event, billed as the time to advice the Foundry on the most relevant organism they shouldminvest in. Market it such that no one would dare miss the meeting and use a criteria driven voting process to identify what orgnaism? What level of development should be pursued? What feedstocks etc. this will enable you to select the organism and processes for development that will benefit the most potential industrial users.



Comments

1. Have a mechanism for deteriming if a company is in line with the foundary's interest.
2. conceriege = single point of contact
3. Needs to be a long term consortium
4. Quarterly meeting of the board
   4.1. alternate between in person and web
5. Is a website enough? No - what about an annual meeting open beyond just an advisory board - yes
6. Annual checklist to make sure still relevant
7. Still have a website...just not the only method of communication.
   7.1. make meeting minutes, etc. available
8. make available to all different sizes of companies
9. Look at the way a project is managed (operational side)
10. Build trust between labs
11. Single point of accountability

Objective and Tasks

- "Early and often" industry engagement
- Shorten the market transformation timeline by 50% over status quo
  - Develop strategic partnerships with industry to create market pull for synthetic biology tools and expertise
    - Identify barriers to industry adoption of synthetic biology technologies; develop strategies to overcome these barriers
      - Emphasis on precompetitive R&D
    - Develop a resource for the community of stakeholders; access to National Lab IP
      - Actively manage relationships and interactions
    - Establish metrics to measure impact on industry and greater bioeconomy
    - Explore models for future public-private partnerships

2

Comments

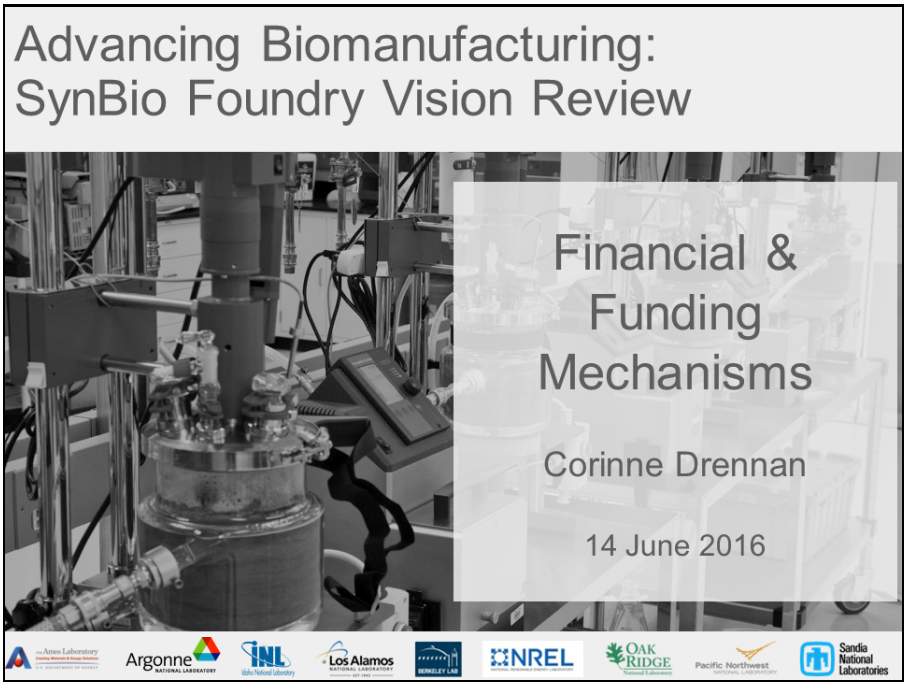
1. Establish firm relationships with industry stakeholders
2. Reword bullet three - the technology is not valuable - advancing the technology is what is valuable

Examples

- Recent BETO-funded biofuels consortia:
  - NABC (National Advanced Biofuels Consortium)
  - NAABB (National Alliance for Advanced Biofuels and Bioproducts)
- Current multi-lab project:
  - Co-Optimization of Fuels & Engines (Co-Optima)
    - Market Transformation Team
- Future model: SEMATECH, e.g.

Comments

1. For either NABC and NAABB does any industry partner provide funding or is all it from the DOE? Related has any technology that was developed in NABC and/or NAABB been commercialized?
2. Strategic and tactical advisories
3. Great suggestion Total



Standard Contracting Mechanisms

Technology Transfer Mechanisms at DOE Facilities

U.S. DEPARTMENT OF ENERGY

Agreement	Use	Funding	Subject Inventions	Generated Data	U.S. Competitiveness	Cost	Highlights
Cooperative Research and Development Agreement (CRADA)	Collaborative research between DOE Labs and public and/or private entities for the mutual benefit of the parties	Private and/or Federal funds	Lab and Participant may elect their own inventions and Participant has right to negotiate exclusive license to Lab inventions	Protected for up to 5 years	Products embodying IP resulting from CRADA shall be manufactured substantially in the U.S.	Lab and Participant may share costs or Participant pays 100% funds-in	✓ Collaborative research ✓ 5 year data protection ✓ Designed for multi-party collaborative research
Strategic Partnership Projects (SPP)	Work for businesses and other non-federal entities using highly specialized or unique DOE facilities, services or technical expertise	Private funds	Sponsor may elect title to Subject Inventions[1]	Protected as Sponsor's proprietary data w/limited exceptions[1,2,3]	U.S. Preference: Sponsor agrees not to grant any party exclusive right to use or sell products embodying Subject Inventions in the U.S. unless products are manufactured substantially in the U.S.	Sponsor pays full cost recovery	✓ Sponsor typically retains right to elect title to subject inventions ✓ Generated data treated as proprietary ✓ Option for limited Gov. R&D license[3]
		Federal funds	Lab may elect title to Subject Inventions of the Lab	Unlimited Gov. rights	U.S. Preference (see above)	Sponsor pays full cost recovery	✓ Access to unique facilities and expertise using federal funds
Agreements for Commercializing Technology (ACT)	Work for businesses and other non-federal entities using highly specialized or unique DOE facilities, services or technical expertise	Private funds	Initial title to the designated IP Lead. (ACT Participant or Lab Contractor)	Protected as proprietary data w/limited exceptions[1,2,3]	U.S. Preference (see above)	Participant pays full cost recovery plus additional negotiated compensation to the Contractor	✓ Flexibility for addressing indemnity & adv. payment ✓ Negotiable IP terms ✓ Optional performance guarantee ✓ Option for limited Gov. R&D license[3]
Proprietary User Agreement[4]	User may access designated facilities to conduct its own proprietary research	Private funds	User may elect title to its Subject Inventions	User may protect as proprietary	n/a	User pays approved user rate	✓ Generated data treated as proprietary ✓ Merit based access to unique facilities
Non-Proprietary User Agreement[4]	Non-proprietary research at designated facilities	n/a	Lab and User may elect their own Subject Inventions	Unlimited Gov. Rights	U.S. Preference (see above)	Each party covers own cost	✓ Merit based access to unique facilities

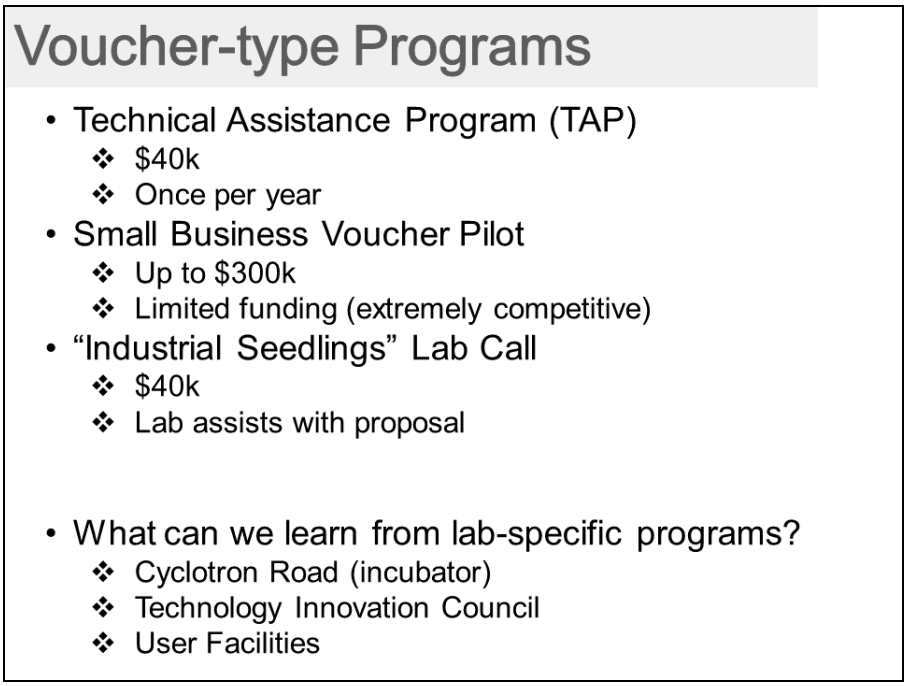
Industry Listening Day – These mechanisms are cumbersome and expensive (e.g., IP premium)

What other options exist?

Comments

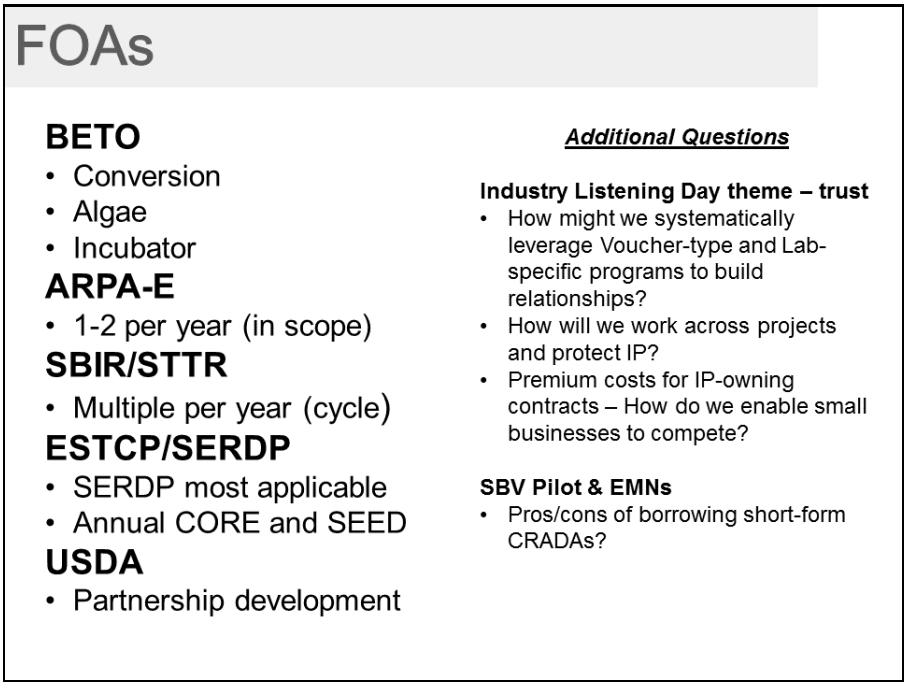
1. Could just use a take it or leave it approach
2. if something is high risk, don't want to spend month making a contract and then lose all of the time and effort if the plan fails.
3. Corinne has seen it standardized on the lab side
4. European public/private partnership (BE-Basic)

5. do you have governmnet marching rights built into contracts?
6. how to align industry and labs? leverage funds in the lab and the private sector; INL feedstock efforts get BETO money but work with industry through cost share.; cost shares are variable (could be in kind and equip, etc);
7. bundle NDA, MTA, and CRADA all in one package (NDA comes before usually)
8. look at European public private partnerships (e.g., Bbasic Now)
9. dealbreaker: one company's IP gets disseminated to another without control.
10. if risk greater than outcome, then normally have staged approach. then, nobody is blocked from doing anything.
11. can we have an umbrella organization navigate natl lab space (IP board) that handles all SynBio Foundry IP
12. want to make as easy as possible for a company to interact
13. having multiple different approaches for interaction will be critical to success at different scales
14. flat rate "try out a license":if like, take the option; in not; go away and give up the $1K
15. one set of document per lab or one document per project with multiple labs?
16. put IP in third-party entity? Could have third party hold IP for all nine national labs



Comments

1. INL could provide insight on PDUs
2. Who owns the proof of concept? Wherever the work is done (lab or DOE)



Comments

1. usda and doe are working on a blanket MOU that will enable more agile partnerships that can be applied here.
2. INL has a bundling mechanism
3. INL user facilities - can do work with other companies, universities, etc. Everyone has a cost share. Level depends on type/size of business.
4. SEMATECH is a non-profit organization of 12 members, and centralizes all of the finances
5. We did something similar in NAABB (related to IP exclusivity)

VISION

1. **Vision: The Synthetic Biology Foundry will unite the capabilities of the national laboratories to develop a robust, agile biomanufacturing platform accessible to researchers across the private and public sectors**
   - 1.1 How about "unite and expand" as there are many private entities that would like to apply synthetic biology foundry capabilities to other desirable industrial hosts such as clostridia species. (Bryan)
     - *1.1.1 agree (valerie)*
   - 1.2 Here (LyinDSC)
   - 1.3 clear interest in having the slides available after the meeting (Phil Laible)

MISSION

1. **Mission: The Synthetic Biology Foundry will integrate industrially-relevant production microbes, advanced tools for biological engineering, and robust processes for integrated biomanufacturing.**
   - 1.1. Does the mission also wish to connect outcomes to scale-up and bioprocess intensification resources such that development to commercialization timelines are significantly shortened also? (Bryan)
   - 1.2. The mission should include new data analysis tools as well as the experimental tools mentioned. (Steve V)

GOAL

1. **Goal: The Synthetic Biology Foundry seeks to develop innovative technologies that are open and accessible to companies, universities, and national laboratories in order to reduce the time and cost of biomanufacturing and to enable a robust bioeconomy.**
   - **1.1 THE NEED FOR A PUBLIC EFFORT**
     - 1.1.1 There are also examples where a company develops expertise in a particular technology but lacks access to other, complementary technologies. (Steve V)
     - 1.1.2 These capabilities DO exist in the private sector, but are often spread among different companies. (Steve V)

FRAMEWORK FOR THE SYNTHETIC BIOLOGY FOUNDRY

1. **Framework for the Synthetic Biology Foundry**
   - 1.1. Figure 1: Keep in mind that once selected, the host strain will have to be improved in conjunction with the pathway as part of the DBTL cycle. (Steve V)
2. **Impact and Three-Year Achievements**
   - 2.1. What are the benchmarks for time to market, cost, efficiency, etc., so that 50% improvement can be demonstrated? (Steve V)
   - 2.2. What is the measure of success for demonstration of a particular target? Just detectable amount of product, or some target titer? Generally it takes much more than one DBTL cycle to get meaningful progress. (Steve V)
3. **Design-Build-Test-Learn Cycle**
   - 3.1. Learn and Test need to work together to design meaningful experiments that will address a particular

question or identify a bottleneck. (Steve V)

3.2. what might work? (Phil Laible)
- *3.2.1. design and learn are the most valuable (Phil Laible)*

3.3. is 9 months a rough estimate? (Phil Laible)
- *3.3.1. could include target selection, TEA. LCA, etc (Phil Laible)*
- *3.3.2. getting done in distributed fasion (Phil Laible)*
- *3.3.3. lots of logistical details that need to be worked out (Phil Laible)*
- *3.3.4. host organism and model/flux generation takes time (Phil Laible)*

3.4. DNA construction eating up too much time? (Phil Laible)
- *3.4.1. month to get design finalized; two months for construction; sequence validation; modifications to host; two months for analysis; 1 month for asessment and learn to inform next round of design (Phil Laible)*
- *3.4.2. new organism or new targets is longer timeline (Phil Laible)*
- *3.4.3. to get to three months, compress significantly. nneds improvment in commercial space for DNA synthesis (Phil Laible)*
- *3.4.4. companyies for synthesis are highly motivated to bring costs down; speed up (Phil Laible)*

3.5. what is a cycle? (Phil Laible)
- *3.5.1. do they run concurrently? (Phil Laible)*
- *3.5.2. can have toxic construct; stop; remake; concurrent asynchronis cycles (Phil Laible)*

3.6. do more difficult hosts impact cycle time? (Phil Laible)
- *3.6.1. doubling time might be critical; also those not onboarded to the same extent (Phil Laible)*
- *3.6.2. use Nth plant model; the nth cycle is quick but the first cycle is likely more cumbersome (Phil Laible)*
- *3.6.3. inherently different cycle times (Phil Laible)*
- *3.6.4. consider liquid screens versus solid? (Phil Laible)*

3.7. consider liquid screens versus solid (Phil Laible)
- *3.7.1. spec sheet for onboarding hosts (Phil Laible)*
- *3.7.2. over time have full metabolic models (Phil Laible)*
- *3.7.3. different tiers of hosts will be treated differently (Phil Laible)*
- *3.7.4. industry feedback relevant; process conditions and first hand experience are CRITICAL! (Phil Laible)*
- *3.7.5. specs for hosts are important (Phil Laible)*
- *3.7.6. industry plays huge role as to what they need (Phil Laible)*

3.8. agree on broad range of hosts (Phil Laible)
- *3.8.1. separations questions as well; can be challenging (Phil Laible)*
- *3.8.2. decentralization MAY BE CRITICAL!!! (Phil Laible)*
- *3.8.3. have evolved hosts or optimized chassis.....????? (Phil Laible)*
- *3.8.4. rational evolution and directed evolution are under consideration (Phil Laible)*
- *3.8.5. combination of them can be important (Phil Laible)*
- *3.8.6. better starting point for target and host engineering (Phil Laible)*
- *3.8.7. expanding the potential hosts will be very helpful (Phil Laible)*
- *3.8.8. when the initial data is in; insertion may be before; integrated platform will need a little time; early stages will allow for license of technology (Phil Laible)*

3.9. at what point envision does industry get involved? (Phil Laible)
- *3.9.1. Process optimization (and then further scale-up) can be very involved and industry is typically very well set up for this. (Michael)*

3.10. role of the labs? (Phil Laible)

3.10.1. a lot of design in LBNL (Phil Laible)
3.10.2. all natl labs contribute to every task (Phil Laible)
3.10.3. some over representation for some labs in certain areas (Phil Laible)
3.10.4. e.g., analytics may be PNNL (Phil Laible)
3.10.5. e.g., NREL and ANL may be TEA and LCA (Phil Laible)
3.10.6. it is not like pet projects being pursued at each national lab. that likely WILL NOT work (Phil Laible)
3.10.7. new technologies will be vetted in context of the DBTL platform (Phil Laible)
3.10.8. e.g., new microfluidic systems may need to be tested within the distributed DBTL network at several different labs (Phil Laible)

3.11. DBTL handoff between labs (Phil Laible)
3.11.1. cultivation and scale down team needed? (Phil Laible)
3.11.2. setting interfaces between centers is critical (Phil Laible)
3.11.3. intially mainly one location has lead in one analytical capability; later it will be slightly more distributed; results need to be consistent! (Phil Laible)
3.11.4. important that whatever working on, then needs to keep scale-up in mind. (Phil Laible)
3.11.5. holistic end-to-end design standpoint (Phil Laible)
3.11.6. test have to be in relevant environment! nothing in isolation (Phil Laible)
3.11.7. not starting from ground zero. FY17 efforts are on going and WE ARE LEARNING (Phil Laible)

3.12. how will this be communicated to community (Phil Laible)
3.12.1. . publications (Phil Laible)
3.12.2. industry outreach (Phil Laible)
3.12.3. work on how to communicate (Phil Laible)
3.12.4. talk on it later but informatic distribution (Phil Laible)
3.12.5. transcriptomics and proteomics can be shareed and make public (Phil Laible)
3.12.6. is there going to be a workshop to help industry work with this data (Phil Laible)
3.12.7. five minute youtube videos for users; might help some; not enough for others (Phil Laible)
3.12.8. we don't have all of the answers today (Phil Laible)

3.13. concerned about sending samples around! (Phil Laible)
3.13.1. what happens during transport (Phil Laible)
3.13.2. sensitive molecules degrade rapidly (Phil Laible)
3.13.3. need some techniques onsite (Phil Laible)
3.13.4. validate with what on site with what happens with more exhaustive measurements (Phil Laible)

3.14. what part is manual; what part is automated? (Phil Laible)
3.14.1. set up with target; many dimensions to design; feedstock; downstream fermentation; bioreactor; IP issues; (Phil Laible)
3.14.2. objective is to integrate as much as possible..... (Phil Laible)
3.14.3. how much resources need to direct to a particular target; sometimes more tolerant to many approaches (Phil Laible)

3.15. groups with specific targets or pathways? (Phil Laible)
3.15.1. lots of different entry points (Phil Laible)
3.15.2. maybe have own enzymes (Phil Laible)
3.15.3. just need to put together strain (Phil Laible)
3.15.4. stage gates for this? (Phil Laible)
3.15.5. cant be doing everything ad hoc (Phil Laible)

3.15.6. if in on beginning, then need to be fairly open source (Phil Laible)
3.15.7. physical or information standards (Phil Laible)
3.15.8. standards around quality assurance (Phil Laible)
3.15.9. do you take their word for material (and sequences, for example)? How much testing to you do when you get a sample? (Phil Laible)
3.15.10. Entry points may be at different levels, e.g. starting at target molecule with retrobiosynthesis or having already pathway or host. Standardization of requirements is critical to manage this. Some of this may be stage-gated (e.g. retrobiosynthesis). (Michael)

3.16. LEARN is a really important part of the cycle; bigger than just one target; learn BIGGER all of the time (Phil Laible)
3.16.1. are there going to be consistent standards (Phil Laible)
3.16.2. relearn and releverage for not just next cycle; next product or pathway!!! (Phil Laible)
3.16.3. don't make the same mistakes over and over (Phil Laible)
3.16.4. do you need to do standard types of tests all of the time to have the best LEARNING (Phil Laible)

3.17. challenge of getting expression in heterologous hosts (Phil Laible)
3.17.1. . can be a huge bottleneck! (Phil Laible)
3.17.2. how will synBIO Foundry handle this (Phil Laible)
3.17.3. having suite of hosts might be a good idea!!! (Phil Laible)
3.17.4. one host might express problematic enzymes better. a host that you would not initially have considered (Phil Laible)

3.18. may not go linearly through DBTL (Phil Laible)
3.18.1. DBTLTLTLTLTLDBTLTLTB, etc (Phil Laible)

3.19. how much will you sequence and verify? (Phil Laible)
3.19.1. be ruthless? (Phil Laible)
3.19.2. probably not, need to know what you are working with (Phil Laible)
3.19.3. don't want to generate noise and reduce predictive power in the future (Phil Laible)
3.19.4. strains evolve; need to know what you are working with (Phil Laible)

3.20. quality versus quantity (Phil Laible)
3.20.1. very clear intermediate points (Phil Laible)
3.20.2. other people need to understand what you did and were trying to accomplish and why you may have bailed on a strategy (Phil Laible)
3.20.3. QC important to make sure data is relevant and as expected to make Learn part in the cycles relevant and not drawing wrong conclusions. (Michael)

3.21. Other agencies: draw from basic sciences (NSF, OS); collaborations are being looked forward to (Phil Laible)
3.21.1. anything different needed in execution (Phil Laible)
3.21.2. need to be in touch at all times (Phil Laible)
3.21.3. foundry and science office need to communicate frequently/constantly; hosts, analytical technology, design technology; how to transition basic science into Foundry labs (Phil Laible)

3.22. what challenges might be most impactful for you? (Phil Laible)
3.22.1. continuing conversation with synthesis providers; larger constructs; lower error; shorter lead times (Phil Laible)
3.22.2. industry input on strain development (Phil Laible)
3.22.3. move away from E. coli and yeasts (Phil Laible)

3.23. link DBTL with basic physiology (Phil Laible)

3.23.1. have constraints on how big this can actually grow (Phil Laible)
3.23.2. can others work with you and collect the other forms of data? (Phil Laible)
3.23.3. might be tackled in host onboarding efforts (Phil Laible)
3.23.4. this efforts could be funded by office of science or NSF or other (Phil Laible)
3.23.5. again, I limited resources (Phil Laible)

3.24. metrics: great for low hanging fruit; not realistic for harder ones (Phil Laible)
3.24.1. how do you handle hard ones? (Phil Laible)
3.24.2. will you be able to handle hard in the future with experience? (Phil Laible)
3.24.3. talk more about efficiency; works for easy and hard (Phil Laible)
3.24.4. tie to host tier (Phil Laible)
3.24.5. you get what you screen for (Phil Laible)
3.24.6. if already dismissed yeast and e. coli, then you might already have trouble making metrics!!! (Phil Laible)
3.24.7. talk about efficiency and you should be fine (Phil Laible)
3.24.8. need MEANINGFUL cycles!!! (Phil Laible)
3.24.9. talk about person hours (Phil Laible)
3.24.10. do you need some sort of measure of improvement? (Phil Laible)
3.24.11. just did not do the hours and get the improvement (Phil Laible)
3.24.12. needs to link to TEA models (Phil Laible)
3.24.13. g/L; yield; and all of those other metrics (Phil Laible)

3.25. don't completely ignore yeasts and e. coli (Phil Laible)

3.26. take existing and increase yield would be industrial relevant to show (Phil Laible)
3.26.1. not many hosts would have those tools; the Foundry could get that data and make it work!!! (Phil Laible)

3.27. side reaction and byproducts and industrial problems! (Phil Laible)
3.27.1. major reactions easy (Phil Laible)
3.27.2. industrial relevance will need to look at side reactions and byproducts; separations; yield; etc (Phil Laible)
3.27.3. stable isotope tracing would enable this analysis; however, scale affects this in ways that are not predicable (Phil Laible)
3.27.4. contaminants and other (Phil Laible)

3.28. what media? industrial media? not clean (Phil Laible)
3.28.1. will be dirty hydrolysates (Phil Laible)
3.28.2. feedstock will come out of the TEA (Phil Laible)
3.28.3. mainly when shift to the commodity chemicals side (Phil Laible)

4. Needed Capabilities to Improve DBTL for Industrial Processes

4.1. Host organism: Also critical are hosts that can consume particular carbon sources of interest. Introducing new substrate utilization pathways into model organisms is difficult, so better to put heterologous product pathways into natural substrate utilizers. (Steve V)

4.2. Industrial considerations: envision the DBTL cycle in the context of an industrial process. Use real world fermentation raw materials from industrial providers (no Sigma or ultrapure components), no yeast extract/peptone or undefined components. Use DE95 (or cellulosic sugars) instead of ultrapure glucose monohydrate. All of these aspects will be helpful to both increasing the value of the technology developed (i.e., requires less development time on the part of the user) and increases the probability of industrial relevance (Jeffrey Dietrich)

5. Standing up the SBF

5.1. At least one of the hosts should be a model organism (E. coli or S. cerevisiae) so progress on the molecule targets can progress even if there are initial challenges with the new host onboarding. (Steve V)

6. Design Technologies

6.1. What about host organism design tools? e.g. COBRA, OptKnock, OptForce, etc. (Steve V)

7. Build Technologies

7.1. As well as forward genetic screens, directed manipulations may be needed to improve the host. (Steve V)

8. Test Technologies

8.1. Be sure to include routine extracellular product/byproduct analysis in this section, in addition to omics data. Keep in mind that significant analytical throughput capacity will be needed to handle the high-throughput culturing. Also important is fermentation process data (sparge rates, off gas analysis, etc.) as well as tracking all the metadata for the experiments. (Steve V)

8.2. Consider standardization of the analytical protocols/methods used (HPLC, GC-MS, ICP-MS). First, this provides a detailed view of what a 3rd party user can expect in terms of byproduct composition (and allows the carbon balance to be closed). Second, this shortens future development time (i.e, by decreasing the need to develop new analytical techniques, identify unknown byproducts, etc). This will also facilitate delivery of an integrated process by providing downstream purification scientists with detailed information about the composition of the fermentation medium. (Jeffrey Dietrich)

9. Learn Technologies

9.1. Machine learning may be able to interpolate to predict production within a range for which we already have data, but will it enable us to extrapolate to higher productivities? Are there literature examples where pure statistical analysis (without taking into account biological meaning) have guided work leading to improved production metrics? (Steve V)

10. Host Onboarding

10.1. Host selection criteria (Michael)

10.1.1. Feedstock is important as it dictates TEA (costs) and LCA. Should consider waste feestocks (e.g. biomass syngas, CO2, glycerol) that have negative value or GHG emissions. Consider including an autotroph and/or phototroph. (Michael)

10.1.2. Focus on unlocking capabilities not accessible and complementary with E. coli/yeast (extreme pH, thermophile, anaerobic, reaction mechanisms not possible due to cofactors). (Michael)

10.1.3. Decentralized concept could actually be an advantage (e.g. separate sporulating strains from non-sporulating strains; specialized foundries, e.g. for anaerobes?) (Michael)

10.1.4. Strains optimized for flux through a certain metabolic node would be of interest. Such improved chassis may come from refinement of hosts after first cycles. (Michael)

10.1.5. Consider evolution to start with a better chassis strain for further cycles. (Michael)

10.2. If you are a hood ornament on a car, you should expect to be hit by bugs (Phil Laible)

10.3. What metabolic properties/traits are needed in new host(s)? (Phil Laible)

10.3.1. Interest in autotrophy was expressed. (Phil Laible)

10.3.2. *extremeophiles might assist with separations as could 'distill' away product without killing the* production strain (in some cases) (Phil Laible)

10.4. What are the desired energy sources? Priorities? (Phil Laible)

10.5. RE: Current hosts -- What improvements are needed? (Phil Laible)

10.6. With respect to new host onboarding, what would an 'ideal' industry-gov't partnership look like? (Phil Laible)

10.7. What kind of work is needed for companies to trust/onboard new organisms and processes? (Phil Laible)
10.8. What scale(s) must be achieved to be industrially relevant? (Phil Laible)
10.8.1. Big enough to know if impurities from feedstocks and/or side reactions and byproducts will be a significant hurdle with the production strategy (Phil Laible)
10.8.2. it is thought that ~100 L is the absolute minimum (Phil Laible)
10.9. What is the ideal molecular toolbox? (Phil Laible)
10.10. What is the minimum criteria for a new 'functional' host? (Phil Laible)
10.11. What works (or doesn't work!) about the proposed plan? (Phil Laible)
10.11.1. Need a Tier Zero category and discussion around it. Might get alternative support for Tier Zeros from Office of Science or NSF (and maybe DARPA) (Phil Laible)
10.12. How can this effort be most impactful over business as usual? (Phil Laible)
10.13. Discussion following session mentioned that DARPA 'electrofuels' strategies may be ones that could be considered in the future by the consortium. There may be merit to some of these approaches that produce biofuels and other bioproducts from electricity and carbon in the environment. (Phil Laible)

11. Process Integration and Scaling

11.1. why just one hydrolysate? (Phil Laible)
11.1.1. expand later (Phil Laible)
11.1.2. simpify (Phil Laible)
11.1.3. standardize across labs (Phil Laible)
11.1.4. TEA and LCA done (Phil Laible)
11.2. do we need to test other hydrolysates? (Phil Laible)
11.2.1. important to know NOW (Phil Laible)
11.2.2. something has to be constant or you have variables all over the place! (Phil Laible)
11.2.3. why corn stover? should this continue to be the default? (Phil Laible)
11.2.4. wood is available nationally, except in southwest (Phil Laible)
11.2.5. feedstock is a larger programmatic BETO question (Phil Laible)
11.2.6. how 'bout gaseous feedstocks? (Phil Laible)
11.3. will cataloged pedigree be part of the Foundry? (Phil Laible)
11.3.1. may already have been distributed (Phil Laible)
11.3.2. 3-4 super sacks at a time 300-400 lbs per sack (Phil Laible)
11.3.3. will lignin serve as an adsorbent? (Phil Laible)
11.4. are we worried about lignin serving as an adsorbant? (Phil Laible)
11.4.1. can't store pretreated stuff; enzymatic hydrolysis on site (Phil Laible)
11.5. what would be better than corn stover (focusing on sugars for a short time) (Phil Laible)
11.5.1. don't use feedstocks from startups (Phil Laible)
11.5.2. too early on in technology development (Phil Laible)
11.5.3. rumatic sugar is not standardized yet (Phil Laible)
11.5.4. processed corn stover may be the best and gauranteed more consistent as all of the rest (Phil Laible)
11.6. will hosts be tested on hydrolysates? (Phil Laible)
11.6.1. YES. This is on onboarding criterion. (Phil Laible)
11.7. any reason to consider a secondary standardized feedstock? (Phil Laible)
11.7.1. just need hot water. would be very consistent. get hemicellulose. don't get unusual side products. might be of value. (Phil Laible)

11.7.2. would we be limited to hardwoods for wood of choice; with softwoods get all C6; avoid C5 problem. (Phil Laible)
11.7.3. could be considered if want to reasonably standard (Phil Laible)
11.7.4. waste is good thing to start with? or mixed feedstocks? (Phil Laible)
11.7.5. Use OCC? Old correagated (sp?) cardboard? (Phil Laible)
11.7.6. in the end, need standardized. (Phil Laible)
11.7.7. maybe outside the auspices of Foundry to test other feedstocks (Phil Laible)

11.8. emphasis is that we need a second real-world high-impact feedstock (Phil Laible)
11.8.1. low costs syrups from milling.....for example (Phil Laible)
11.8.2. could be part of the host onboarding process (Phil Laible)
11.8.3. tell OS or NSF that we have unmet host onboarding and feedstock flexibility needs (Phil Laible)
11.8.4. can we leverage other separations efforts (Phil Laible)

11.9. feedstocks have impacts on sooooo many parts of the process. (Phil Laible)
11.9.1. gave yeast extract swap example (Phil Laible)
11.9.2. for example, can't use hydrolysates to make a polymer (Phil Laible)
11.9.3. you end up with hydrolysate components in the polymer!!! no question (Phil Laible)

11.10. bioproducts that enable biofuels (Phil Laible)
11.10.1. doesn't have to be done in the same facility! (Phil Laible)
11.10.2. reduces risk; facilitates getting financing (Phil Laible)

11.11. SynBio Foundry can't do everything! (Phil Laible)
11.11.1. worth doing clean feedstocks versus dirty (Phil Laible)
11.11.2. dirty will have to work with different downstream process..... (Phil Laible)
11.11.3. feedstocks may be cheap but separations may kill you! (Phil Laible)
11.11.4. Low value will require CHEAP at every step. High value targets gives one more flexibility (Phil Laible)

11.12. seems like need a very strong relationship with large companies early on! (Phil Laible)
11.12.1. missing expertise for scaling, etc.; a good story, even if it takes a decade, is worth it; how do we get the good stories and knowledge gained from them? (Phil Laible)

11.13. some stuff might be outside scope (Phil Laible)
11.13.1. again, we can't do everything (Phil Laible)
11.13.2. have to solve all problems to be successful (Phil Laible)

11.14. what is correct scope? (Phil Laible)
11.14.1. have to solve all problems to be successful? (Phil Laible)
11.14.2. industrial opportunities and risk that are allowed are changing landscapes (Phil Laible)

11.15. what needs to be done to deRISK a production situation? (Phil Laible)
11.15.1. what are key things that have to be done in order to move forward? (Phil Laible)
11.15.2. thinking of it as a technology push (Phil Laible)
11.15.3. this is where labs and DOE sit (Phil Laible)
11.15.4. needs to be labDOE partnership with industry to get market pull (Phil Laible)
11.15.5. needs LOTS of conversations to overcome this dilemma (Phil Laible)
11.15.6. solving is not straightforward (Phil Laible)
11.15.7. don't want to end with two industry workshops (Phil Laible)
11.15.8. advisory board will help -- but only to a certain degree (Phil Laible)
11.15.9. need to have the correct mindset (Phil Laible)

MANAGEMENT OF THE SBF

1. Management Structure

1.1. how to communicate with industry? (Phil Laible)
- *1.1.1. funding for first 3-4 years will be government (at least the bulk) (Phil Laible)*
- *1.1.2. consider some sort of mechanism by which present info that is being created and the status of individual efforts to industry (in unbiased form) (Phil Laible)*
- *1.1.3. one on one and membership only won't work as well. (Phil Laible)*
- *1.1.4. have more industry days with progress updates (Phil Laible)*
- *1.1.5. this is a way to add value from industry (more or different than monetarily) (Phil Laible)*
- *1.1.6. concierge is too DOE-ish; industry won't engage if any mechanism for being disadvantaged (Phil Laible)*
- *1.1.7. might be a competitive part that is coming in the short term (Phil Laible)*
- *1.1.8. is a website enough? some say NO; most people want a face to face conference; beyond advisory board meeting; open to followup discussions (Phil Laible)*
- *1.1.9. make sure industrial relevant checkpoints every year (Phil Laible)*
- *1.1.10. website tools are very important (Phil Laible)*
- *1.1.11. website could have host onboarding information and could share standardized datasets there (Phil Laible)*
- *1.1.12. important that smaller companies have the opportunity to use all of the tools (Phil Laible)*

1.2. how to interact with small companies? (Phil Laible)
- *1.2.1. usually a question of costs (Phil Laible)*
- *1.2.2. what licensing available, etc. make SBIR exceptions? (Phil Laible)*

1.3. what has worked well in the other examples? (Phil Laible)

1.4. what did not work well? (Phil Laible)
- *1.4.1. carbon fiber from ORNL; consortium fell part as no fundamental mechanism to keep it going (Phil Laible)*
- *1.4.2. consortium could fall apart from lack of funding (Phil Laible)*
- *1.4.3. need long term confidence; needs to have legs to stand for some period of time (Phil Laible)*

1.5. advisory board issues (Phil Laible)
- *1.5.1. consensus seem that quarterly is minimal; three by phone and one in person (Phil Laible)*

1.6. advisory board tasks (Phil Laible)
- *1.6.1. individual projects (Phil Laible)*
- *1.6.2. project management; operational side (Phil Laible)*
- *1.6.3. there are best at operations and could be used most valuably there (Phil Laible)*

1.7. other ways of industrial engagement? (Phil Laible)
- *1.7.1. workshops after some results have been obtained; intensive workshop to see how it is working (Phil Laible)*
- *1.7.2. hard to tell now without seeing some accomplishments (Phil Laible)*
- *1.7.3. what results would you like to see at a workshop: shorten timescale from prototype to production (would like to know more about that process; how?) (Phil Laible)*
- *1.7.4. industry validation of technology and process (Phil Laible)*
- *1.7.5. could see work on new hosts with own hands (Phil Laible)*
- *1.7.6. would build trust between labs and industry (Phil Laible)*
- *1.7.7. there are programs with funding available for this purpose. (Phil Laible)*

1.8. two levels of input from industry: strategy and technical (Phil Laible)

1.8.1. may need to split into two different boards (Phil Laible)
1.8.2. one more strategic (Phil Laible)
1.8.3. one more technical (Phil Laible)
1.8.4. strategic may be more critical (Phil Laible)
1.8.5. technical is needed for adoption of the technology (Phil Laible)
1.8.6. have scientists in residence in several locations (labs and industry) (Phil Laible)
1.8.7. developer's forum discussed by caller-in..... (Phil Laible)

1.9. careful with wording on "market pull for synthetic biology tools'. Wrong. Value brought on by them. (Phil Laible)

1.10. need SPAs (single points of accountabilities) (Phil Laible)

1.11. website: one point of contact but could go throuugh any lab that you would want to go to (Phil Laible)

1.11.1. need someone that can navigate the national lab landscape; doesn't need to manage every simgle relationship (Phil Laible)

CONCLUSION

1. Conclusion: This document outlines a framework of operations for the Synthetic Biology Foundry of national labs, including the R&D foci for the SBF and a proposed management structure. This document is informed by discussions with industry stakeholders, EERE staff, and staff from the national labs. Further engagement with industry, other Federal government stakeholders, academic researchers, and the community will be sought out to improve the framework.

1.1. General Challenges/Comments/Needs:

1.1.1. Engage Industry "Early and Often" to build trust, interest, etc
1.1.2. Concentrate on Interface(s) between DBTL
1.1.3. Once developed, provide an method to Educate Industry on how to use the new tools, plus a means to distribute and communicate Host improvements. Similarly, clear Reporting mechanism needed for "Intermediate" process successes. (i.e, goal is to build pathway from A to B to C, but if bottleneck found at B, report the progress
1.1.4. Metrics for Success- Time improvement for Host Onboarding and/or DBTL cycle may not be best- Try to capture the magnitude of Impact
1.1.5. Work to Understand Biproducts and Side Reactions (colorants, odorants, contaminating organisms)- (general seen at scale up but not at lab scale)
1.1.6. Corn Stover may not be the best choice as the standard hydrolysate/crude substrate across the foundry. Woody plant material, or switchgrass may be more appropriate.

1.2. HOST Onboarding DISCUSSION

1.2.1. Interest in "Evolved" hosts vs. GMO (Provide choices/alternatives within a host)
1.2.2. Heterologous expression is an issue, need methods to overcome and/or evaluate if a transgene is functional a priori. Ideally, a Host bank would be utilized to hedge/mitigate
1.2.3. Must consider the Regulatory framework/issues as a part of the onboarding Criteria.
1.2.4. Start with a list of organsisms currently in use, and build out
1.2.5. Evaluate 'zero value' feedstocks as substrates
1.2.6. Organisms that grow at Low pH, high temperature

1.2.7. Contamination tolerance /sterility minimization
1.2.8. General support for critical 'metabolic nodes' (beach head molecules as targets)
1.2.9. Interest in alternate Bacillus host development
1.2.10. Syngas utilization, phototrophy, autotrophy requested
1.2.11. Ideal to onboard phototrophs that have mixotrophic potential
1.2.12. Scale evaluation- 100-200L scale appears to be most predictive of full scale production (1000+ liters) while 5-10 liter tests are not as informative- General need to effectively simulate at small scale- screen with variables seen at larger scale (i.e. larger temperature/pH swings due to larger reactors) at lab scale to improve screening efficacy
1.2.13. RE: Scale up- suggested as a way to interface/partner with industry (let industry test/evaluate the scale up).
1.2.14. Host characterization/Selection Criteria could include evaluation of a small panel of standard hydrolysates (corn stover, switchgrass, woody plant, etc). May not be time to create a DBTL cycle for each substrate but baseline knowledge will be useful for future development efforts.
1.2.15. Strains should originate from the U.S. (avoid international rights claims) (Shawn Starkenburg)

OVERALL FEEDBACK

1. Overall Feedback

1.1. societal impacts; benefit to people; avoid backlash
1.1.1. avoid complications that can be parallelizing
1.2. willing to talk any time; line of communication open with program managers now
1.2.1. can leverage things, when possible

2. Only the appendix has the comments from the March workshop. Maybe implement some of those things into the vision document.

3. can we take advantage of living foundries (DARPA)

4. NUGOYO (sp?) agreement impact

4.1. if strain taken from another country, then you need to know the origin of it; otherwise, the country will need to be compensated
4.1.1. strains might need to originate in the united states; complicate use by other countries; avoid compensating other countries
4.2. industry wants involved early in strain and target selection

5. At what point is it most appropriate to fully engage with industry? What needs to be established?

5.1. Early on - input on strain and target selection
5.2. early an often
5.3. case by case basis?
5.4. mechanism that starts small and leads to something bigger later

6. at what point do we engage in partnering; what is needed to be shown? appropriateness of timing

7. engage industry forums to disseminate results